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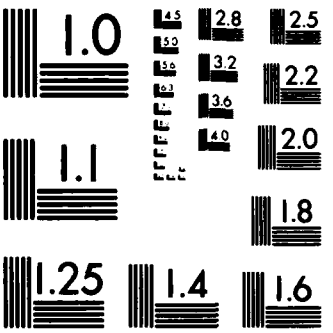

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Annual Report

(1 September 1983 - 31 July 1984)

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John E. Donelson, Ph.D.

September 1984

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Using starting material supplied by Dr. Klaus Esser at the Walter Reed Army Institute for Research, we have constructed two different cDNA librariesfrom the mRNAs of "day 5" trypanosomes which have been shown to be expressing predominately metacyclic variable surface glycoproteins (M-VSGs). In these libraries have been identified cDNA clones that contain the coding sequences for 4 of the 12 - 15 different M-VSGs expressed by metacyclic Trypanosoma brucei rhodesiense. These cDNAs have been sequenced and used as probes to characterize the corresponding chromosomal genes. They have also been subcloned into E. coli expression vectors so that the bacteria synthesize large quantities of fusion proteins expressing the M-VSG epitopes. It is anticipated that these fusion proteins can be used for immunological studies. As a corollary to the M-VSG project, we are also investigating several aspects of the DNA rearrangements that are associated with the expression of VSGs during the bloodstream stages of the parasite infection. We have determined the complete sequence of a 1.35 kb repeat sequence that contains the coding sequence for

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19. Abstract (Con't)

the 35 nucleotides that are found at the 5'-termini of all VSG mRNAs. Fragments containing this sequence are now being used as probes to investigate the transcription of these 35 nucleotides in isolated trypanosome nuclei under a variety of conditions. During the coming year we plan to continue to expand both the metacyclic VSG project and the transcription studies.

ANNUAL PROGRESS REPORT

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"Characterization of the Variable Antigen Genes Expressed by Trypanosoma brucei rhodesiense During Metacyclic Stage and in the Bloodstream"

1 September 1983 - 31 July 1984

September 1984

John E. Donelson, PhD
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Two general research projects are being conducted using funds from this contract. They are described here under the headings of (I) The Metacyclic VSG Gene Project and (II) The Bloodstream VSG Gene Project. Good progress has been made on both projects as documented under (III) Publications During 1984. I anticipate that progress will continue at a good pace during the coming second year of this contract.

(I). The Metacyclic VSG Gene Project.

As has been well documented by many review articles during the past few years, African trypanosomes go through several developmental stages in their intermediate host, the tsetse fly. The final stage in the fly is called the metacyclic stage. Metacyclic organisms are injected into the bloodstream of the mammalian host during the tsetse fly bite. Dr. Klaus Esser, our collaborator, and his colleagues at Walter Reed Army Institute of Research have shown that each metacyclic parasite expresses on its surface one of about 12 - 15 different variable surface glycoproteins (VSGs). Once in the bloodstream, these metacyclic VSGs (M-VSGs) continue to be expressed for about 5 days after which there is a switch to the first bloodstream VSGs (B-VSGs). During this time the parasites undergo further metabolic changes and multiply with a doubling time of about 3 hours. Trypanosomes have several hundred different B-VSG genes. After the 5th day of infection, one of the battery of several hundred B-VSG genes begins to be transcribed. This gene will be expressed for several days in a given organism and its progeny until this lineage is either nuetralized by the host immune system or a few of the progeny switch to the expression of another B-VSG. This phenomenon of sequential switching from one B-VSG to another is called antigenic variation and results in the successive waves of parasitemia that are characteristic of trypanosome infections.

The molecular basis at the DNA and RNA level for antigenic variation has been the subject of intense investigation by 6 - 10 different labs around the world during the past 5 years, including our own. These studies have revealed a complex series of gene rearrangements that are associated with the switch from the expression of one B-VSG gene to another. Furthermore, it has been shown that sometimes intragene rearrangements also occur which can give rise to the expression of new genes for immunologically-distinct B-VSGs. This means that trypanosomes possess the ability to synthesize a virtually infinite number of B-VSGs from the several hundred basic copy B-VSG genes. Therefore, it seems very unlikely that it will be possible to develop a vaccine against the bloostream parasites. However, since metacyclic trypanosomes express only a limited subset of 12 - 15 different VSGs, it may be possible to develop a vaccine against the initial trypanosome infection. For this reason, Dr. Esser and I are concentrating on the identification and characterization of the M-VSG genes in Trypanosoma brucei rhodesiense, a human pathogen.

Dr. Esser has identified monoclonal antibodies against all of the M-VSGs of one particular isolate of this parasite. Unfortunately, it is not possible to obtain sufficient metacyclic parasites from infected tsetse flies to conduct biochemical or recombinant DNA studies on these organisms. Each fly contains about 50,000 metacyclic parasites but about 10e9 are required to isolate enough M-VSG or mRNA for characterization. However, it is possible to obtain sufficient quantities of parasites still expressing M-VSGs if they are collected from experimental animals infected by metacyclic organisms 4 - 5 days previoulsy. We have done this and constructed cDNA libraries from the mRNA of these day 5 parasites. These cDNA libraries were screened for those clones containing M-VSG coding sequences by several procedures. The two most effective identification procedures were differential RNA hybridization in RNA dot blots and expression of M-VSG epitopes by the recombinant bacteria. It has taken us over a year to confirm these cDNA identifications but to date we have cloned and characterized cDNAs for 4 different M-VSGs (1). We have determined all or part of these cDNA sequences and found that the basic primary structures of the M-VSGs are very similar to that of B-VSGs. The same C-terminal homology regions are present, N-terminal signal peptides and C-terminal hydrophobic tails occur and potential sites of glycosylation appear at approximately the same locations.

We have also used these cDNAs as hybridization probes to investigate the genomic environment of the M-VSG genes. We find that the gene for each of the four M-VSGs is located near a different telomere and that there are no corresponding intrachromosomal gene copies. This is different than all, or almost all, telomere-linked B-VSG genes where there is at least one similar or identical isogene copy also present within the chromosomes themselves. Another curious feature of the M-VSG genes is that they are not flanked by an upstream region of several kb containing repetitive sequences lacking restriction

sites, i.e., the so-called "barren" regions upstream of telomere-linked B-VSG genes (1). Rather, the M-VSG genes are preceded by regions that possess a conventional random distribution of restriction sites. This may suggest that the M-VSG genes are less prone to rearrangement than are the B-VSG genes but at this point it is not clear. Furthermore, these initial experiments have not revealed a clear-cut molecular basis for the differential expression of the M-VSG genes, i.e., why only the M-VSG genes are expressed at the metacyclic stage and the several hundred B-VSG genes are not.

Therefore, we have cloned two of the telomere-linked M-VSG chromosomal genes plus their flanking regions and are in the process of also cloning the two other M-VSG chromosomal genes. We hope that a more detailed analysis of these cloned genes will reveal clues about the mechanism of their differential regulatin. In particular we plan to determine the sequences of about 2 kb upstream of these genes in anticipation that there may be common regulatory signals in these regions.

In addition we are also cloning the M-VSG coding sequences into E. coli expression vectors so that we can obtain bacteria strains that synthesize large quantities of the M-VSGs themselves for immunological studies (2). Our first attempts in this effort utilized an expression plasmid in which fusion proteins between E. coli beta-galactosidase and the inserted sequences were made. This was accomplished by digesting the cloned cDNA fragments with Bal-31 to randomized their ends and then inserting fragments into a restriction site within the coding sequence for beta-galactosidase. Several such fusion proteins have been identified on SDS-acrylamide gels by staining indicating that they comprise several percent of the total protein synthesized by the bacteria. In one case, Dr. Esser has shown very nicely that the fusion protein binds in a Western blot to a monoclonal antibody directed against a M-VSG. This demonstrates that the fusion protein possesses the epitope that was recognized by the monoclonal. Therefore, we should be able to use an affinity column for beta-galactosidase to purify the fusion protein from all of the other E. coli proteins so that it can be used for specific immunization studies. These experiments will be under way during the coming year.

In summmary, we are now past the most difficult portion of this project, i.e., unambiguously identifying several M-VSG cDNAs. I anticipate that we will be able to make nice progress on all aspects of this project during the next few years.

(II). The Bloodstream VSG Gene Project.

An important component of this study is to compare and contrast the M-VSG genes with the B-VSG genes. Only in this way can the features unique to M-VSG expression be determined.

Therefore, we are also characterizing several B-VSG genes and some interesting aspects of their differential transcription.

One project involves the investigation of what happens to the expressed B-VSG gene when trypanosomes are ingested by the tsetse fly. Other labs have shown that the B-VSG ingested by the fly is often the first non-M-VSG to appear when the fly infects another animal. This suggests that the gene for the ingested B-VSG may remain at the expression site but in an inactivated state during passage through the fly. Upon re-introduction into the bloodstream this gene is then re-activated. To test this model, we are using a bloodstream clone of T. rhodesienese called WRATAT 1. This is the clone that was used to infect flies from which were derived the M-VSGs that we are studying. We also have clones of WRATAT 1 which appeared in the first non-metacyclic parasitemia after the fly infection. We are in the process of obtaining a cDNA probe of the WRATAT 1 VSG to be used in a series of genomic Southerns. We anticipate that these Southern hybridizations will reveal any rearrangements associated with the inactivation and re-activation of this gene. At the moment we have constructed the appropriate cDNA library and have tentatively identified several clones that code for the WRATAT 1 VSG. Therefore, it should be possible to conduct the experiments in the near future.

Another project involves a characterization of the coding region for the 35 nucleotides that are found at the 5'-ends of all VSG mRNAs including those for the M-VSGs. It turns out that this coding sequence is found within a 1.35 kb tandem repeat which we have cloned and completely sequenced (3). About 100 nucleotides downstream from the coding sequence is a very dA/T rich region containing many internal repeats which we think are important in controlling transcription of this region. In very recent experiments we have been able to acheive transcription of this region in isolated trypanosome nuclei using radioactive UTP and a filter binding assay. This opens the door for a careful analysis of how the independently transcribed 35-mer is attached to the 5'-ends of the VSG mRNAs and many other trypanosome mRNAs as well. We anticipate that experiments will contribute to our understanding of the regulation of trypanosome transcription.

In a series of related experiments we are attempting to biochemically resolve the 3 conventional RNA polymerase activities from trypanosome extracts. Dr. Tony Weil's lab is adjacent to mine here at the University of Iowa. He is an expert on eukaryotic RNA polymerases and is helping us with these experiments. Interestingly, we have been unable to separate the 3 activities (RNA polymerases I, II and III) from trypanosome extracts for reasons that are not understood. Only one peak of activity occurs when all of the conventional column separation procedures are used. This single peak of activity displays a sensitivity to alpha-amanitin that does not correspond to any of the conventional activities. Although it may be premature to speculate at this time, it is possible that trypanosomes have a different sort of RNA polymerase activity than other eukaryotes

which is related to the unusual presence of the conserved 35-mer at the 5'-ends of (perhaps) all trypanosome mRNAs. We will continue to pursue these experiments during the coming year.

As a corollary to the above effort to resolve the 3 RNA polymerase activities, we decided to investigate the structure of the trypanosome 5S rRNA gene, a representative class III gene that is transcribed by RNA polymerase III in conventional eukaryotes. We isolated the 5S rRNA band from an acrylamide gel and labelled it for use as a probe to identify clones of the 5S gene. After some effort we identified a cloned 760 bp fragment and sequenced across it. A portion of its sequence matches the consensus sequence for 5S rRNA indicating that we had indeed obtained the desired clone. We have now immobilized this fragment on filters for use in studying synthesis of the 5S rRNA under different conditions. We hope that these experiments will help to resolve the question of how many RNA polymerase activities are present in trypanosomes and what their biological functions are.

In summary, we have identified several interesting aspects of B-VSG gene expression that can be approached experimentally and are related to our main goal of studying the M-VSGs and the expression of their genes.

(III). Publications During the Year 1974.

1. Lenardo, M., Rice-Ficht, A., Kelly, G., Esser, K. and Donelson, J. (1984). Characterization of the Genes Specifying Two Metacyclic Variable Antigen Types in Trypanosoma brucei rhodesiense. Proc. Natl. Acad. Sci. USA (in press).

2. Lenardo, M., Brentano, S. and Donelson, J. (1984). Expression of Antigenic Regions of a Trypanosome Variable Surface Glycoprotein in E. coli Using Bal-31 Nuclease Digestion. Nucleic Acids Research 12: 4637-4652.

3. Dorfman, D. and Donelson, J. (1984). Characterization of the 1.35 kb Repeat Containing the Conserved 35 Nucleotides at the 5'-Terminal VSG mRNAs in Trypansoma brucei. Nucleic Acids Research 12: 4907-4920.

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Lagrimini, L., Brentano, S. and Donelson, J. (1984). A DNA Sequence Analys8s Package for the IBM Personal Computer. Nucleic Acids Research 12: 605-614.

Johnson, B., Hill, G. and Donelson, J. (1984). The Maxicircle of Trypanosoma brucei Kinetoplast Encodes Apocytochrome b. Mol. Biochem. Parasit. (in press).

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