The 222- to 234-Kilodalton Latent Nuclear Protein (LNA) of Kaposi’s Sarcoma-Associated Herpesvirus (Human Herpesvirus 8) Is Encoded by orf73 and Is a Component of the Latency-Associated Nuclear Antigen

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Kaposi’s sarcoma (KS)-associated herpesvirus or human herpesvirus 8 (KSHV/HHV8) is the likely cause of KS and primary effusion lymphomas or body cavity-based lymphomas (BCBLs). A latency-associated nuclear immunofluorescence antigen (LANA) (D. H. Kedes, E. Operskalski, M. Busch, R. Kohn, J. Flood, and D. Ganem, Nat. Med. 2:918–924, 1996; S. J. Gao, L. Kingsley, M. Li, W. Zheng, C. Parrarivici, J. Ziegler, R. Newton, C. R. Rinaldo, A. Saah, J. Phair, R. Detels, Y. Chang, and P. S. Moore, Nat. Med. 2:925–928, 1996) and a 222- to 234-kDa nuclear protein (LANA) (S. J. Gao, L. Kingsley, D. R. Hoover, T. J. Spira, C. R. Rinaldo, A. Saah, J. Phair, R. Detels, P. Parry, Y. Chang, and P. S. Moore, N. Engl. J. Med. 335:233–241, 1996) have previously been described in BCBL cell lines by immunofluorescence and Western blotting techniques, respectively. To identify the viral gene(s) encoding this antigen(s) we screened a cDNA library from HBL-6 cells, a B-cell lymphoma cell line persistently infected with KSHV/HHV8, with KS patient sera. One set of positive clones contained the 3′ end of orf73, as well as the complete orf72 and orfK13, and another set contained the 5′ end of orf73. Comparison of cDNA sequences with the KSHV/HHV8 genomic sequence revealed a splice event, occurring upstream of orf73. Immunoaffinity purified antibodies to a recombinant carboxy-terminal fragment of the orf73-encoded protein showed the characteristic speckled nuclear immunofluorescence pattern of LANA and reacted with the 222- to 234-kDa LNA on Western blots. Expression of full-length orf73 in bacteria and COS7 cells reproduced the LNA banding pattern. Immunohistochemistry on cases of nodular KS revealed that orf73/LNA is expressed in the nucleus of KS spindle cells. These findings demonstrate that orf73 encodes the 222- to 234-kDa LNA, is a component of LANA, and is expressed in KS tumor cells.

Karla’s sarcoma (KS)-associated herpesvirus (KSHV), or human herpesvirus 8 (10), is found in all forms of KS (2, 5, 11, 13, 21, 27, 35), in AIDS-associated body cavity-based lymphoma (BCBL) or primary effusion lymphoma (7), and in a proportion of cases of multicentric Castleman’s disease (38). Detection of KSHV in peripheral blood of human immunodeficiency virus (HIV)-infected individuals correlates with and predicts the onset of KS (28, 42). Serological studies also indicate that infection with KSHV is common among HIV-infected homosexuals (16, 17, 19, 22, 37), who develop KS only exceptionally (4), but not among other HIV risk groups such as patients with hemophilia and intravenous drug users (16, 17, 19, 22, 37), who develop KS only exceptionally (4, 31). KS can be transmitted sexually (19). Immunofluorescence antigen (LANA) (D. H. Kedes, E. Operskalski, M. Busch, R. Kohn, J. Flood, and D. Ganem, Nat. Med. 2:918–924, 1996) and a 222- to 234-kDa nuclear protein (LANA) (S. J. Gao, L. Kingsley, M. Li, W. Zheng, C. Parrarivici, J. Ziegler, R. Newton, C. R. Rinaldo, A. Saah, J. Phair, R. Detels, Y. Chang, and P. S. Moore, Nat. Med. 2:925–928, 1996) have previously been described in BCBL cell lines by immunofluorescence and Western blotting techniques, respectively. To identify the viral gene(s) encoding this antigen(s) we screened a cDNA library from HBL-6 cells, a B-cell lymphoma cell line persistently infected with KSHV/HHV8, with KS patient sera. One set of positive clones contained the 3′ end of orf73, as well as the complete orf72 and orfK13, and another set contained the 5′ end of orf73. Comparison of cDNA sequences with the KSHV/HHV8 genomic sequence revealed a splice event, occurring upstream of orf73. Immunoaffinity purified antibodies to a recombinant carboxy-terminal fragment of the orf73-encoded protein showed the characteristic speckled nuclear immunofluorescence pattern of LANA and reacted with the 222- to 234-kDa LNA on Western blots. Expression of full-length orf73 in bacteria and COS7 cells reproduced the LNA banding pattern. Immunohistochemistry on cases of nodular KS revealed that orf73/LNA is expressed in the nucleus of KS spindle cells. These findings demonstrate that orf73 encodes the 222- to 234-kDa LNA, is a component of LANA, and is expressed in KS tumor cells.

KSHV establishes a latent infection in KS spindle cells as well as BCBL cell lines (6, 14, 32, 43). KSHV genes known to be expressed in KS tissue include that for K12, a putative 60-amino-acid (60-aa) hydrophobic protein and the type D cyclin homolog (v-cyclin) encoded by orf72, the chemokine receptor homolog encoded by orf74, a nuclear RNA (T1.1), and the viral homologs of bcl-2 and MIP-1b (9, 39, 40, 43). Whereas most spindle cells express mRNA for K12 and v-cyclin, only a small proportion of spindle cells, which may undergo lytic replication, express the nuclear RNA (T1.1), v-bcl2, and v-MIP-II (39, 40).

Sera from KSHV-infected individuals react in immunofluorescence with a nuclear antigen in latently infected BCBL cell lines which is characterized by a speckled immunofluorescence pattern (17, 19, 29, 37) and has been termed latency-associated nuclear antigen (LANA) (19). The antigen was first discovered by using the Epstein-Barr virus (EBV)-KSHV coinfected cell line HBL-6 (29) but has since been used as a highly specific antigen for preparations from EBV−/KSHV+ BCBL cell lines...
On Western blots of nuclear extracts from the BC-1 cell line, patient sera also react with a 222- to 234-kDa doublet band, termed latent nuclear antigen (LNA) (16). Detection of Western blot-defined LNA correlates closely with LANA reactivity (17). However, whether LNA is identical with LANA has so far not been established. Both the latent immunofluorescence assay (IFA) and the LNA Western blot assay are very specific assays for the detection of KSHV antibodies (16, 17, 37).

In view of the important role that nuclear antigens (EBNAs) of EBV (human herpesvirus 4) a gamma-1 herpesvirus related to KSHV, play in B-lymphocyte transformation, we attempted to identify the viral gene(s) encoding LANA and/or LNA. We show here that LNA is encoded by orf73 of KSHV and that antibodies to this protein produce the speckled nuclear immunofluorescence pattern typical of LANA. This suggests that orf73/LNA is at least a component of, if not identical with, LANA. We also show that orf73/LNA is expressed in the majority of spindle cells of nodular KS lesions and thus is likely to play a role in the pathogenesis of KS.

**MATERIALS AND METHODS**

Construction and screening of HBL-6 cDNA library. A cDNA phage library of the unstimulated HBL-6 cell line (8, 35) was constructed in the ZapII vector according to the manufacturer’s (Stratagene’s) instructions and amplified. We screened approximately 10⁸ phage on nitrocellulose filters, using a conventional protocol, with a pool of five sera from endemic KS (HHV-negative) patients. Patient sera were diluted 1:500 (each serum was diluted 1:250) in phosphate-buffered saline (PBS)–3% dried skim milk (Marvel)–0.1% Triton X-100–0.05% sodium azide–10% fetal calf serum and incubated with filters for 1 h at room temperature. Filters were washed in PBS–0.1% Tween 20, followed by an alkaline phosphatase–conjugated goat-anti-human immunoglobulin G (IgG) (1:7,500; Seralab) and nitroblue tetrazolium with 5-bromo-1-chloro-3-indolylphosphate (BCIP) (Promega) as substrate. Positive phage were rescreened twice by the line phosphatase-conjugated goat-anti-human immunoglobulin G (IgG) (1:7,500; Seralab) and nitroblue tetrazolium with 5-bromo-1-chloro-3-indolylphosphate (BCIP) (Promega) as substrate. Positive phage were re-screened twice by the line phosphatase-conjugated goat-anti-human immunoglobulin G (IgG) (1:7,500; Seralab) and nitroblue tetrazolium with 5-bromo-1-chloro-3-indolylphosphate (BCIP) (Promega) as substrate. Positive phage were re-screened twice by the line phosphatase-conjugated goat-anti-human immunoglobulin G (IgG) (1:7,500; Seralab) and nitroblue tetrazolium with 5-bromo-1-chloro-3-indolylphosphate (BCIP) (Promega) as substrate. Positive phage were re-screened twice by the line phosphatase-conjugated goat-anti-human immunoglobulin G (IgG) (1:7,500; Seralab) and nitroblue tetrazolium with 5-bromo-1-chloro-3-indolylphosphate (BCIP) (Promega) as substrate.

Northern blot, but its expression can be induced by treatment with sodium butyrate or dibutyrate phorbol ester (25, 26, 36).

Immunohistology on KS lesions. To investigate the expression of orf73/LNA in KS tissue, we used tissues from eight nodular- and two early (patch-plaque) stage KS biopsies, embedded in paraffin. Sections of 5 μm were cut, and the paraffin was removed by sequential immersion in xylene and ethanol. Sections were then treated for 15 min in a microwave oven (high power setting) and endogenous peroxidase was blocked by treatment with methanol–3% hydrogen peroxide. Affinity-purified antibodies to orf73 and orf65, diluted 1:5 in Tris-buffered saline (TBS)–5% BSA, were added for 1 h at room temperature. Following two washes in TBS, a biotin-conjugated anti-human IgG antibody (Dako), diluted 1:200 in TBS–5% BSA, was added for 30 min. This was followed by treatment with streptavidin-conjugated horseradish peroxidase (Dako), diluted according to the manufacturer’s instructions, and diaminobenzidine (0.5 mg/ml)–0.1% H₂O₂ for 7 min. To identify endothelial tumor (spindle) cells, adjacent sections were stained with a monoclonal antibody to CD34 (QB-END 10; Seratech, Oxford, United Kingdom) by the same procedure, except that biotin-conjugated rabbit anti-mouse antibody (Dako) was used as the second antibody.

Immunoscreening and mapping of cDNA clones. HBL-6 is a BCBL-derived B-cell line which is dually infected with KSHV and EBV (15, 33), isolated from the same tumor as BC-1 (8) and previously shown to be serologically indistinguishable from BC-1 (29). KSHV is tightly latent in this cell line, and no expression of the orf26 minor capsid gene is detectable by Northern blot, but its expression can be induced by treatment with sodium butyrate or dibutyrate phorbol ester (25, 26, 36).

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We screened approximately 10⁶ clones of a cDNA library, constructed from uninduced HBL-6 cells, using a pool of sera from patients with endemic KS, and obtained five clones which could be grouped into two sets (Fig. 1). Sequence analysis of the inserts revealed that two clones (clones 5 and 7) (Fig. 1), representing the first set, started at different points within orf73, extended to the end of orf73 and beyond through orf72 (v-cyclin) and another downstream open reading frame (orfK13), and terminated at nucleotide 122,066 of the published KSHV sequence (33), 28 bp downstream of an AAT AAA polyadenylation site at nucleotide 122,094. These two clones included a poly(A) tail (Fig. 1). At its 5′ end, located within the internal repeat of orf73, the sequence of clone 5 was identical with the genomic BC-1 sequence. However, clone 7 lacked nucleotides 124,578 to 124,595, which correspond to a repeat subunit (AGGGATTTAGGGAGGTGG on the coding strand, translated as ELEEEVE). Clone 7 may therefore be derived from a viral episome with a different repeat structure. Another clone (clone 15), representing the second set, started at nucleotide 127,866 of the published sequence (33) upstream of orf73 and ended at nucleotide 125,094 within the internal repeat region of orf73. In addition to containing the sequence matching that of KSHV, this clone contained a stretch of cellular sequence which had provided its poly(A) tail, suggesting that this clone arose from a recombination event or from a KSHV genome which had integrated into cellular DNA. This clone did not contain nucleotides 127,313 to 127,811 of the genomic KSHV sequence. This segment of genomic sequence has typical intron boundaries (GTGAGT..TTGTCAG) on the coding strand and appears therefore to have been spliced out of the mRNA encoding orf73 (Fig. 1).

Antibodies to orf73 react with LANA in immunofluorescence and the 222- to 234-kDa LNA in Western blots. The first open reading frame within cDNA clones 5 and 7 corresponded to that of orf73 and was contiguous with the β-galactosidase-encoding region of the phagemid vector. This construct encoded the complete orf73 sequence except for the first 4 aa, fused with 6 His residues, followed by an enterokinase cleavage site in the amino terminus. An XhoI/HindIII fragment of KSHV mRNA encoding orf73, corresponding to nucleotides 122,789 to 127,285 of the published KSHV sequence (33), was cloned into the XhoI and HindIII sites of pBlueScript-KS (Stratagene). An XhoI/XbaI fragment was obtained from this construct and inserted into the XhoI and XbaI sites of pcDNA3.1/His B (Invitrogen) to obtain a mammalian expression vector. This construct encodes the complete orf73 sequence except for the first 4 aa, fused with 6 His residues, followed by an enterokinase cleavage site in the amino terminus. An XhoI/HindIII fragment was obtained from this construct and inserted into the XhoI and HindIII sites of pBlueScript-KS (Stratagene). This construct encodes the complete orf73 sequence except for the first 4 aa, fused with 6 His residues, followed by an enterokinase cleavage site in the amino terminus.
encoding gene in the λZap vector. This suggested that the carboxy-terminal 256 aa of orf73, which were included in both clones 5 and 7, contained an epitope(s) reactive with patient sera and that the protein encoded by orf73 was expressed in latently infected BCBL cell lines. We expressed the last 212 aa of orf73 as a fusion protein with dihydrofolate reductase in *Escherichia coli*. This region of orf73 was chosen because it does not extend into the internal repeat region within orf73 (Fig. 1), which is predicted to encode a highly negatively charged protein sequence. When used as an antigen in an enzyme-linked immunosorbent assay, the purified recombinant orf73 fragment reacted with 12 of 18 serum samples (66%) from endemic KS (HIV-negative) cases and 1 of 25 sera (4%) from matched controls. Therefore, not all latent immunofluorescence-reactive patient sera react with this recombinant protein, most likely because it represents only a small segment of the protein encoded by orf73.

To establish the relationship between the orf73-encoded protein, the immunofluorescence-defined LANA, and the Western blot-defined 222- to 234-kDa LNA of KS, we affinity-purified orf73-specific human antibodies from patient sera on the 212-aa recombinant orf73 fragment. These orf73-specific antibodies produced the speckled nuclear staining pattern characteristic of LANA also seen with KS patient sera on BCBL-1 cells (Fig. 2), which are only infected with KSHV (17), and on HBL-6 cells (not shown), which are latently infected with both KSHV and EBV (15). Antibodies affinity-purified on recombinant orf52 and orf65 proteins did not stain these two (uninduced) cell lines (Fig. 2).

Affinity-purified antibodies to the recombinant carboxy-terminal orf73 fragment, but not to orf52 protein, also reacted with the high-molecular-weight doublet band (LNA) in nuclear extracts of BC-1 and BC-1 cells (Fig. 3A and B) which is recognized by most sera from KS patients (Fig. 3C and reference 16). The same antibody preparation did not recognize any bands in nuclear extracts from P3HR-1, an EBV-infected B-cell line (Fig. 3A). Interestingly, the molecular weight of the LNA doublet was slightly higher in BC-1 cells than in BC-1 cells (Fig. 3). PCR amplification with primers flanking the internal repeat region within orf73 revealed that this region is approximately 300 bp longer in BC-1 cells than in BCP-1 cells (not shown), providing a possible explanation for the size difference between the LNA doublets in these cell lines. These findings show that the Western blot-defined 222- to 234-kDa LNA protein is encoded by orf73, and that it is either identical with, or at least a component of, immunofluorescence-defined LANA.

**Expression of orf73 in *E. coli* and COS7 cells gives rise to LNA.** We cloned orf73, derived from BC-1 cells (8, 33), into the mammalian expression plasmid pcDNA3.1/His B and the bacterial expression plasmid pTrcHis (Invitrogen), such that aa 5 to 1162 of orf73 were preceded by six His residues and an enterokinase cleavage site. Western blotting of both bacterial and transfected COS7 lysates with KS patient sera, but not control patient sera, reproduced the high-molecular-weight doublet pattern of the LNA (Fig. 4). Affinity purification of the His-tagged orf73 protein from bacterial extracts (Fig. 4) provides final confirmation that orf73 encodes the LNA and indicates that the much-larger-than-expected apparent molecular mass of the orf73 protein (expected molecular mass, approximately 135 kDa) is likely due to its primary structure rather than posttranslational modification. However, the size difference between the orf73 expressed in mammalian cells and that in *E. coli* (Fig. 4) indicates that posttranslational modification does occur.

orf73/LNA is expressed in KS endothelial tumor (spindle) cells. Using the affinity-purified human antibodies to orf73 and orf65, we investigated the expression of these two viral proteins in KS tissue. As shown in Fig. 5A and B, orf73/LNA was expressed in KS nodules in cells with a spindle-shaped morphology, which on parallel sections stained strongly with the endothelial marker CD34 (Fig. 5D) and therefore met accepted criteria for KS spindle cells, the endothelial tumor cells of KS lesions. Expression of orf73/LNA was confined to the nucleus, in keeping with its expression in lymphoma cell lines (Fig. 2 and 5). Antibodies to orf65 never produced any nuclear

![Diagram](image-url)
staining (Fig. 5C). We found expression of orf73/LNA in eight of eight cases of nodular KS, but not in two early-stage (patch or plaque) KS lesions. Expression in spindle cells of nodular KS lesions was variable, with some nodules showing orf73/LNA expression in the majority of spindle cells (Fig. 5A) and others showing only a few positive cells (Fig. 5B). We cannot exclude the possibility that our affinity-purified human antibodies may have failed to detect weak expression of orf73/LNA in some cells or that different fixation conditions may have been responsible for these differences. Whether expression of this protein is limited to fully developed KS lesions therefore remains to be investigated further. We also noted that within the same biopsy sample, some spindle cells showed a diffuse nuclear staining (Fig. 5A) whereas the staining in others was confined in a dot-like pattern to subnuclear domains (Fig. 5B), reminiscent of the coarse staining seen by immunofluorescence.
in some lymphoma cells. Whether these two staining patterns correspond to different stages of infection or reflect an association of orf73/LNA with different nuclear structures at different points in the cell cycle is currently under investigation. With the exception of plasma cells reacting with the anti-human conjugate antibody, no cytoplasmic staining was ever seen, nor did orf73-specific antibodies stain any of a series of control biopsy specimens, including tonsil, colon, pancreas, and adenocarcinoma specimens.

**DISCUSSION**

We show in this report that KSHV orf73 encodes a previously described high-molecular-weight nuclear protein (LNA) (16) and is a component of the LANA defined by immunofluorescence (17, 19). This conclusion is based on our observations (i) that two sets of cDNA clones extending into orf73 were recognized by patient sera, (ii) that human antibodies affinity purified on a recombinant orf73 fragment give rise to the typical nuclear immunofluorescence pattern of LANA and react with the 222- to 234-kDa LNA, and (iii) that orf73 expression plasmids encoding aa 5 to 1162 of orf73 in COS7 cells and *E. coli* produce the characteristic 222- to 234-kDa Western blot doublet in nuclear extracts. We also show that orf73/LNA is expressed in the nuclei of KS spindle cells. This is in agreement with a recent report (9) that RNA sequences spanning the orf73 region can be detected in KS lesions by reverse transcription-PCR (RT-PCR). However, neither the expression of the corresponding protein nor the nature of the cell expressing it could be established by this technique.

The characteristic Western blot pattern of LNA is a doublet of approximately 226- to 234-kDa in HBL-6 or BC-1 cells and a slightly lower molecular mass (approximately 220- to 230-kDa) in BCP-1 cells. This size difference seen in different cell lines is probably related to the length of the internal repeat region in orf73, which differs in these two cell lines and also in KSHV obtained from different patients (13a). There are several possible explanations for the presence of a doublet band in both cell lines: the observation that expression of a cloned orf73 sequence in COS7 cells and bacteria (Fig. 4) also produces this doublet suggests that it results from differences in posttranslational modifications of the orf73 protein sequence. Differences in posttranslational modification between mammalian cells and *E. coli* may also explain why the molecular weight of the orf73/LNA doublet in *E. coli* is slightly lower than that in COS7 or BC-1 cells (Fig. 4). Alternatively, proteolytic cleavage at a particular site may produce the doublet pattern or explain the weaker bands also seen in Fig. 3A. The sequence difference between two of our cDNA clones (clones 5 and 7) in the internal repeat region of orf73 could suggest that a different arrangement of repeat subunits on individual episomes within a cell line may occur, but is unlikely to be responsible for these two major protein bands. Although it would be an unlikely explanation in view of the doublet nature of orf73 expressed in *E. coli*, we have investigated the possibility of alternative splicing within the internal repeat region, using RT-
PCR and several combinations of primers flanking this domain, but have failed to find any evidence for this (not shown). The apparent molecular mass of all orf73/LNA bands on sodium dodecyl sulfate (SDS)-polyacrylamide gels is much higher than that predicted on the basis of the primary sequence (predicted molecular mass, approximately 135 kDa); this is likely due to the highly negatively charged stretch of amino acid residues encoded by the internal repeat region of orf73, resulting in the aberrant migration of the protein on SDS-polyacrylamide gels. Interestingly, the transforming STP protein of herpesvirus saimiri (HVS), which contains a collagen-like internal repeat domain, also migrates during SDS-polyacrylamide gel electrophoresis as a doublet band with a molecular weight much higher than expected (18).

Our findings suggest that both the carboxy-terminal region of orf73 (which we expressed as a recombinant protein fragment) and its amino-terminal domain (included in clone 15) are recognized by patient sera. Our carboxy-terminal recombinant fragment reacted with only approximately 60% of sera from latent IFA-positive endemic-KS patients, suggesting that individual sera may recognize different epitopes in orf73/LNA. A full-length recombinant protein is at present being evaluated.

KSHV infects endothelial and spindle cells of KS lesions (6), and an abundant latent transcript with the potential to encode a small 60–aa hydrophobic protein (orfK12) is expressed in the majority of KS spindle cells (39, 40). In addition, a nuclear RNA is strongly expressed in a few spindle cells which also express mRNA for a minor capsid protein and may therefore undergo lytic replication (39). The expression of orf73/LNA in the majority of KS spindle cells of nodular KS lesions and uninduced BCP-1 and HBL-6 cells (Fig. 2 and 5) suggests that it is expressed in persistently infected cells. Persistently infected BCBL-derived cell lines also express a viral cyclin homolog (orf72), and a homolog of interleukin-6 (orfK2) (30, 34). The expression of mRNA for orf72 has also been noted in KS lesions (9). Therefore, the role of KSHV in promoting the growth of endothelial cells most likely involves the expression of at least two nuclear proteins (orf73/LNA and orf72/Cyclin), one of which (v-cyclin) can interact with and phosphorylate the Rb cell cycle checkpoint control protein (12).

orf73 is located in a genomic region which is poorly conserved between the different gamma herpesviruses sequenced so far (1, 3, 23, 41). Among the gamma-2 herpesviruses whose sequences have been published, i.e., KSHV (33), HVS (1), equine herpesvirus 2 (41), and bovine herpesvirus 4 (23), equine herpesvirus 2 lacks an orf73 homolog. The orf73 homolog of bovine herpesvirus 4 does not contain the internal repeat domain present in KSHV and HVS (23). The corresponding genomic region of EBV, a gamma-1 herpesvirus, is much larger than that in gamma-2 herpesviruses and contains the extended BamHI W repeat and adjoining regions encoding EBNA-LP and EBNA-2 (1, 3). EBNA-2 and EBNA-LP mRNAs are extensively spliced in the BamHI W repeat. We however failed to detect any major splicing within the internal repeat of orf73 by RT-PCR in BCBL cell lines. The spliced nuclear staining pattern of orf73/LNA is reminiscent of that seen with EBNA-2 and EBNA-LP and may reflect the association with subnuclear structures. Whether there are functional similarities between orf73/LNA and EBNA-2 or EBNA-LP, which are the earliest proteins to be expressed in newly infected B lymphocytes and are essential for B-lymphocyte transformation (20), is presently under investigation.

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REFERENCES


25. Miller, G., M. O. Rigsby, L. Heston, E. Grogan, R. Sun, C. Metroka, J. A. Miller, G., M. O. Rigsby, L. Heston, E. Grogan, R. Sun, C. Metroka, J. A.


39. Schulz, T. F. Unpublished observations.


