Invasive and Metastatic Potential Induced by ras-Transfection into Mouse BW5147 T-Lymphoma Cells

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ABSTRACT

Noninvasive, nonmetastatic BW5147 T-lymphoma cells were transfected with the activated human c-Ha-ras oncogene and were examined subsequently for the acquisition of invasive properties in vitro and of metastatic potential in vivo. It was found that several transfectants harboring the ras gene had become invasive in vitro, as assessed in hepatocyte cultures, and metastatic after tail vein injection into syngeneic AKR mice. The induced level of both invasive and metastatic potential appeared to depend on the level of expression of the transfected ras gene. Those transfectants exhibiting an elevated level of ras expression, mostly cells containing a high copy number of the ras gene, showed the highest invasiveness (up to 30-fold increase) and produced widespread metastasis in all mice tested. Transfectants with a low level of ras expression were less invasive and formed metastases in a few mice only, limited to a few organs or even to a single deposit in one organ. Untransfected BW cells, control transfected cells without the ras gene, and ras transfectants that did not express the gene were noninvasive and nonmetastatic. No changes in number of ras gene copies were found between isolated metastases and the transfectants from which they were derived. However, RNA analysis of the cells from the isolated metastases revealed similar, as well as elevated or diminished levels of ras transcription when compared to the corresponding cell lines prior to injection, suggesting that a persistent high expression of the ras gene is not necessarily needed for the independent growth at the secondary site. Our results indicate that the activated human ras oncogene may confer metastatic potential onto lymphoid tumor cells, probably due to the induction of invasiveness.

INTRODUCTION

Various types of oncogenes have been identified whose activated forms are implicated in the development of cancer (for reviews, see Refs. 1 to 3). However, it is not known whether the activation of particular oncogenes is essential for the induction of invasiveness and metastatic potential. It has been reported that among the ES lymphoma tumor cell variants an activated Ki-ras oncogene was detectable only in the metastatic variant (4). In contrast, among five separate metastatic deposits originating from a single melanoma patient only one contained an activated ras gene (5). Furthermore, no difference in the relative level of the ras-encoded p211 was found between primary and metastatic human colorectal tumors (6). However, other investigators observed high levels of the ras p21 by immunohistochemical staining in deeply invading adenocarcinoma of the colon, intermediate levels in superficially invasive carcinomas, and low or undetectable levels in normal colonic mucosa or benign colon tumors (7).

By transfection experiments it has been shown that the metastatic potential of tumor cells may be modified by the introduction of the activated ras oncogene. Enhanced metastatic capacity of mouse mammary carcinoma cells was observed after transfection of the c-Ha-ras oncogene (8). In addition, ras-transfected NIH 3T3 cells produced lung metastases in 100% of nude mouse recipients (9-11) and were metastatic upon injection into the embryonic chick (12) in contrast to untransfected 3T3 cells. These transfectants secreted increased levels of type IV collagenase and had acquired the capacity to invade human amnion basement membrane in vitro (11) as well as cultures of embryonic chick heart fragments (13).

In previous work we have shown that fusion of highly invasive activated normal mouse T-cells with tumorigenic but noninvasive, nonmetastatic mouse BW5147 T-lymphoma cells yields highly invasive T-cell hybrids which are also highly metastatic in syngeneic mice (14, 15). Apparently, properties of normal T-cells, quite likely including those responsible for invasiveness, caused the BW cells to become metastatic. In the present study we have examined whether the ras oncogene is also able to influence invasive and metastatic potential in the same BW5147 T-lymphoma cells. To this end, BW5147 cells were transfected with a plasmid containing both the neomycin resistance gene and the c-Ha-ras oncogene derived from the human EJ bladder carcinoma cell line. BW cells transfected with the neomycin gene only were taken along as controls. Transfectants were examined for the presence and expression of the cotransfected ras gene and investigated for changes in invasiveness in vitro as assessed in hepatocyte cultures and in metastatic potential in vivo after i.v. injection in syngeneic AKR mice.

It was found that only transfectants harboring and expressing the introduced ras gene had become invasive and metastatic. Moreover, the induced level of both invasiveness and metastatic potential appeared to depend on the degree of amplification and level of expression of the transfected ras gene. Our results suggest that the ras oncogene may confer metastatic potential onto lymphoid tumor cells by the induction of invasiveness.

MATERIALS AND METHODS

Cells and Transfection Assay. BW5147 T-lymphoma cells (arisen in an AKR mouse) were grown in RPMI 1640 medium supplemented with 10% NCTC (Flow), 10% fetal calf serum, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 0.5 mM sodium pyruvate, 1 mM oxaloacetic acid, 0.05 mM 2-mercaptoethanol, and 0.2 IU/ml bovine insulin (RPMI1640 medium, modified from Ref. 16; all reagents from Sigma, St. Louis, MO). DNA transfections were carried out as described recently (17). Prior to DNA transfection, actively growing BW cells (5 x 106) were centrifuged and resuspended in 10 ml Dulbecco’s modified Eagle’s medium supplemented with 10% newborn calf serum. Transfection was performed in 75-cm2 Falcon flasks (5 x 105 cells/10 ml) by the addition of 1 ml DNA-calcium phosphate precipitate containing 20 μg high-molecular-weight normal human carrier DNA with or without 2 μg plasmid DNA. Cells were transfected with the neomycin resistance gene only or in combination with the c-Ha-ras gene derived from the human bladder carcinoma (EJ) using the plasmids pRSV-neo (18) and pRSV-neo-EJ, respectively. The pRSV-neo-EJ was constructed by inserting the 6.6-kilobase BamHI fragment of pEJ in the BamHI sites of the pRSV-neo vector and was kindly provided by J. W. G. Janssen. After 16 h of transfection the cells were centrifuged, and the medium was replaced by RPMI medium (20 ml). After 6 h of growth the cells were resuspended in fresh RPMI medium containing 2 mg/
ml G418 to select for transfectants. Cells were distributed in 16-mm wells (500,000 cells/well), and the medium was partly replaced every 4 days by fresh RPMI* medium containing G418. By G418 selection all cells except those carrying the RSV-neo DNA were killed. After 2 to 3 wk, growth of cells resistant to Geneticin (G418, GIBCO) was observed in some wells. These transfectants were expanded in RPMI* medium plus G418 for further analysis.

DNA Analysis. DNA from control and test cells was isolated as described before (17), digested with the appropriate restriction endonuclease, electrophoresed in a 0.6% agarose gel, and transferred onto nitrocellulose filters. Filters were prehybridized for at least 2 h at 63°C in 3x SSC, 10x Denhardt's (bovine serum albumin (2 mg/ml)-Ficoll 400 (2 mg/ml)-polyvinylpyrrolidone (2 mg/ml)), 0.1% SDS, denatured salmon testes DNA (50 µg/ml), and 10% dextran sulfate and hybridized for 16 h at 63°C in hybridization mix with 0.3P-labeled probes (5 x 10^6 dpm/ml). For hybridization the pRSV-neo plasmid (18) or the 6.6-kilobase BamHI c-Ha-ras-1 fragment was used as a probe to assess the presence of the respective DNA sequences in the transfectants. Filters were washed 3 times for 30 min in 3x SSC-0.1% SDS at 63°C and once in 1.1x SSC-0.1% SDS. Hybridization was detected by autoradiography for 1 to 7 days at -70°C using Kodak X-1 films and Ilford intensifying screens.

RNA Analysis. Cells (2 x 10^6) were washed 3 times with cold phosphate-buffered saline and lysed in 10 ml of ice-cold 6 M urea/3 M LiCl (19) and immediately homogenized with an Omnismixer at 1 min at maximum speed. The homogenate was stored for 48 h at 4°C and then centrifuged for 1 h at 9500 rpm at 4°C (Beckman Model J2-21 centrifuge and Model JA-20 rotor). Pellets were dissolved in 4.5 ml of 10 mM Tris-HCl (pH 7.5)-2 mM EDTA-0.2% SDS. Next, 0.5 ml of 5 M NaCl were added, and the RNA was deproteinized by two phenol extractions and precipitated with ethanol. Polyadenylated RNA was selected by binding to oligo(dT) microcellulose column. Bound material was then eluted 3 times with 1 ml of 10 mM Tris-HCl (pH 7.6)-1 mM EDTA-0.05% SDS, ethanol precipitated, and then dissolved in sample buffer for electrophoresis.

RESULTS

Characterization of the BW5147 Transfectants. To obtain cell lines harboring the ras gene, BW5147 cells were transfected with the neomycin resistance gene in combination with ras (pRSV-neo-EJ). As a control BW cells were transfected with the neomycin gene (pRSV-neo) only. Transfectants were selected in the presence of 2 mg G418/ml culture medium. No major changes in morphology, growth rate, or tumorigenicity upon s.c. injection in syngeneic AKR mice were observed among the G418-resistant transfectants and BW parent cells. A panel of cell lines induced by pRSV-neo-EJ and pRSV-neo alone was analyzed for the presence and expression of the introduced DNA sequences. All transfectants harbored pRSV-neo sequences. In most cases the intact 5.7-kilobase BamHI pRSV-neo fragment was present as well as various additional hybridizing fragments (not shown). DNAs of the various transfectants were then analyzed for the presence of the cotransfected human c-Ha-ras gene. Hybridization was performed with the human purified 6.6-kilobase BamHI c-Ha-ras gene fragment in the presence of competitor DNA (pRSV-neo) to ensure that transfected pRSV-neo sequences would not interfere with interpretation of the data. In virtually all neo-EJ-transfected cell lines human c-Ha-ras DNA sequences were present which were not detectable in untransfected BW cells (Fig. 1, Lane BW) and cells transfected with the neo gene only (Fig. 1, Lanes BW(neo) 1 to 3). In most of the lanes on the left filter in Fig. 1 a faint endogenous mouse c-Ha-ras fragment with a molecular size of 3.5 kilobases was retained due to less stringent conditions of washing. Approximately 50% of the transfectants contained the intact human 6.6-kilobase c-Ha-ras fragment that was also seen in human control DNA (Fig. 1, Lane HUM). In most of these transfectants various additional hybridizing fragments of different sizes were present as a result of loss and gain of restriction enzyme sites upon transfection. In some transfectants such rearranged ras gene copies were present only. In the transfectants BW(neoEJ) 4, 12, and 13, many copies of the ras gene were detected probably as a result of amplification of the transfected gene (compare with two gene copies of human control DNA, Fig. 1).

Next, we examined the cell lines for transcription of the integrated ras genes. To this end, poly(A)+-RNAs were examined for molecules reactive with the 6.6-kilobase c-Ha-ras probe (Fig. 2). To be able to compare the amount of poly(A)+-RNA on the filters, the filters were also hybridized with a tubulin probe detecting a 2.0-kilobase tubulin-specific transcript (lower part of Fig. 2). Human ras-specific transcripts, 1.2 kilobases in size, were found in the transfectants BW(neoEJ) 4, 6, 9, 11, 12, and 13. A very low level of ras transcription was seen in BW(neoEJ) 10 and possibly 7 and 8. Comparison of the degree of hybridization among the various transfectants after annealing with the ras as well as the tubulin probe indicates that the level of ras transcription was particularly elevated in the transfectants harboring the amplified ras gene copies, BW(neoEJ) 4, 12, and 13 (see Figs. 1 and 2). Note the weaker tubulin signal of BW(neoEJ) 11 in comparison to BW(neoEJ) 13.

Invasiveness of the Transfectants. The cell lines obtained by transfection with pRSV-neo-EJ and pRSV-neo alone were subsequently examined for changes in invasive potential in vitro.
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Fig. 1. Southern blot showing the presence of human c-Ha-ras sequences in 5 μg BamHI-digested DNAs from BW5147 transfectants. Lane BW, mouse BW control DNA; Lane HUM, human control DNA; Lanes BW(neoEJ)1-11, DNAs of BW cells transfected with the plasmid containing both the neomycin gene and the human c-Ha-ras (EJ) gene; Lanes BW(neo)1-3, DNAs from BW cells transfected with the neomycin gene only. Hybridization was performed with the purified human 6.6-kilobase c-Ha-ras fragment in the presence of competitor DNA (pRSV-neo phis mi.I). Note the multiple copies of c-Ha-ras in BW(neoEJ)4, 12, and 13. The number of integrated ras gene copies was roughly estimated from these filters and included in Table 1.

Fig. 2. Northern blot showing human c-Ha-ras specific transcripts (1.2 kilobases) in the various BW(neoEJ) transfectants as presented in Fig. 1. The filters were hybridized with the purified 6.6-kilobase BamHI human c-Ha-ras fragment and after that with a tubulin probe showing a 2.0-kilobase tubulin specific transcript as an internal quantitative control for the amounts of RNA on the filters (separately shown below). The relative level of c-Ha-ras transcription among the various transfected was estimated from these filters and included in Table 1.

Invasiveness was assessed in primary rat hepatocyte cultures. This assay was chosen because many types of lymphoma cells are in particular highly invasive in liver tissue. In sections of embedded hepatocyte culture fragments that infiltrated cells as well as hepatocyte nuclei were counted. The infiltration index = number of infiltrated cells per 100 hepatocyte nuclei was used as a measure of invasiveness. As shown previously (14, 15), noninvasive nonmetastatic cell lines such as BW5147 have an infiltration index smaller than 10. To facilitate the comparison among the various transfecteds in Table 1, the estimated number of integrated ras gene copies (Fig. 1) and the relative level of ras transcription (Fig. 2) have been included. As shown in Table 1, only the transfecteds expressing the human c-Ha-ras gene had acquired invasive potential. In some transfecteds invasiveness was increased up to 30-fold compared to the BW parental cells. Transfecteds without integrated ras sequences, BW(neo)1 to 3, and transfecteds without detectable expression of the human ras gene were all found to be noninvasive (infiltration index, <10). The highest infiltration indices were >100 and exhibited by the cell lines with many ras gene copies and an elevated level of ras expression, BW(neoEJ)4, 12, and 13 (Table 1). Invasiveness was not or slightly enhanced in transfecteds with low and intermediate levels of ras transcription, BW(neoEJ)11, 6, 9, and 10. These data suggest that the degree of acquired invasive potential depended on the level of expression of the introduced ras gene.

Metastatic Potential of the Transfectants. The transfecteds were subsequently tested for the capacity to produce experimental metastases upon i.v. injection into the tail vein of syngeneic AKR mice. As shown previously (14, 15), BW5147 cells are tumorigenic upon s.c. injection into AKR mice and give rise to large tumors 3 to 4 wk after inoculation but do not form experimental metastases upon tail vein injection. Also in this study the untransfected BW cells failed to produce metastases, and this was also found for the BW(neo) control transfecteds (Table 1). In contrast, the transfectants BW(neoEJ)4, 12, and 13, exhibiting an infiltration index >100 and a high level of ras expression, gave rise to widespread metastasis in all mice tested. Livers were diffusely infiltrated, and their size was up to 3-fold increased. Also the spleens were enlarged while nodular tumor foci were found in the kidneys. Large tumors were also observed in the ovaries. The lungs, however, always had a normal appearance. A similar widespread metastasis pattern was found for the transfecant BW(neoEJ)11, exhibiting an infiltration index of 32. Transfecteds with relatively low levels of ras expression and hardly enhanced invasiveness, BW(neoEJ)6 and 9, or even lacking invasive potential, BW(neoEJ)10, formed in some mice either metastases in a few organs or a single metastasis. The latter were often located in a lymph node. The noninvasive transfecteds, BW(neoEJ)1, 2, 3, 5, 7, and 8 (infiltration index, <10), which did not express the transfected ras gene generally failed to produce metastases. Exceptions were BW(neoEJ)7 and 8 which gave rise to a single metastasis in a few mice (Table 1). ras gene expression was hardly detectable in these two transfecteds (see Fig. 2). Metastasis formation was assessed 6 mo after i.v. injection of $1 \times 10^6$
Table 1  

<table>
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<th>Code transfectants&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Estimated no. of EJ copies&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Relative level of EJ expression (mRNA)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Invasive potential&lt;sup&gt;d&lt;/sup&gt; (infiltration index)&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Metastatic potential&lt;sup&gt;f&lt;/sup&gt;</th>
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<sup>a</sup> BW(neo) and BW(neoEJ) transfectants obtained by transfection of BW5147 with the plasmids pRSV-neo or pRSV-neo-EJ, respectively.  
<sup>b</sup> Estimated from the DNA filters as presented in Fig. 1 showing the presence of human c-Ha-ras sequences in the transfectants.  
<sup>c</sup> Estimated from the RNA filters as presented in Fig. 2 showing ras specific transcription in the BW(neoEJ) transfectants.  
<sup>d</sup> As assessed after 7-h incubation on primary cultures of hepatocytes.  
<sup>e</sup> Infiltration index, number of tumor cells infiltrated per 100 hepatocyte nuclei.  
<sup>f</sup> Metastatic potential as tested upon tail vein injection of 1 X 10^6 transfectants in syngeneic AKR mice. Local, only one single metastasis was found; widespread, metastases were found in various organs as indicated. L, liver; M, mesentery; S, spleen; K, kidney; O, ovaries; R, retroperitoneal. Mice were examined after 6 mo or earlier when they became moribund. Ratio represents the number of mice with metastases/number of mice tested.  
<sup>g</sup> NT, not tested; ND, not detected.  
<sup>h</sup> Values in parentheses, detectable after long exposure times.

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... cells or earlier when the mice became moribund. As shown in Table 1, the shortest latency periods were found for transfectants which caused widespread metastasis, in particular the ones exhibiting high ras expression. In view of the long observation periods it was determined that the arisen metastases originated from the injected transfectants and not from spontaneously arisen AKR lymphomas. Therefore some metastases were isolated and cultured in vitro for further analysis. The resulting cell lines appeared to be still resistant to G418 and to harbor the human ras gene, proving that they were derived from the injected transfectants. DNA analysis of the transfectants and their corresponding metastases revealed no differences in the number of ras gene copies (Fig. 3, left three pairs of lanes). This was found for the transfectants BW(neoEJ)9 and 11 harboring a few ras gene copies only as well as for BW(neoEJ)4 cells containing a high ras gene copy number. RNA analysis of transfectants and cells from the corresponding metastases, however, revealed a large variation in ras specific transcription. In the three metastases studied a similar, an elevated, or a diminished level of ras transcription was found when compared with the corresponding cell lines prior to injection. Apparently, the level of ras transcription is not necessarily stable in the transfectants and may change in time before the metastatic cells can be isolated or alternatively during expansion of the isolated cells prior to analysis.

**DISCUSSION**

This study demonstrates that the capability to invade and to metastasize can be imparted to BW5147 T-lymphoma cells by transfection of the activated human c-Ha-ras oncogene. Only those transfectants which harbored and expressed the ras gene acquired invasive potential as assessed in vitro in hepatocyte cultures, an assay chosen because many types of lymphoma cells are in particular highly invasive in liver tissue. Invasiveness was increased up to 30-fold compared to untransfected BW cells, cells transfected with the neomycin resistance gene only, and ras transfectants that did not express the ras gene. In general, the degree of ras gene expression correlated with the extent of acquired invasive and metastatic potential. Those transfectants exhibiting an elevated level of ras expression, mostly cells containing a high number of ras gene copies, were most invasive and produced widespread metastasis upon tail vein injection, in all syngeneic mice tested. In contrast, transfectants with low or undetectable levels of ras expression were less or noninvasive and formed metastases in only a few mice, limited to a few organs or even to a single deposit in one organ. Our results suggest that the degree of induced invasive and metastatic potential of the BW T-lymphoma cells is related to the level of expression of the introduced ras oncogene.

In previous work we have shown that in vitro fusion of the
same BW5147 T-lymphoma cells and activated normal T-cells gives rise to highly invasive tumor T-cell hybrids which are also highly metastatic (14). By prolonged culturing and subcloning, noninvasive and nonmetastatic hybrids could be selected that had arisen as a result of chromosome segregation (15). Since normal activated T-cells are highly invasive in vitro in contrast to nonactivated T-cells, it was concluded that invasiveness of the normal activated T-cells may be an important property for metastasis formation of the tumor T-cell hybrids. Fusion events leading to metastatic tumor variants have also been shown to occur spontaneously in vivo (for reviews, see Refs. 23 to 25). Thus, properties derived from normal cells may also induce a highly malignant phenotype. Remarkably, it appears that the activities of normal T-cell gene products responsible for invasiveness can be mimicked by the activated ras gene product. It is to be noted, however, that the level of invasiveness of the T-cell hybrids is higher (approximately 5 times) and the latency period shorter (approximately 2 to 3 times) as compared to the ras transfectants with high levels of human ras transcription. The differences in latency periods between the transfecteds and hybrids are not due to differences in growth rate in vitro. Apparently, the ras gene product is able to mimick only to a limited extent the activities of the actual genes responsible for invasive properties of activated T-cells and T-cell hybrids.

Our present results add to previous findings with other cell types in which invasive and metastatic potential was induced or enhanced by transformation of the activated ras gene. ras-transfected mouse mammary carcinoma cells metastasized more readily and to more tissue sites (13, 26). Furthermore, several reports have shown that transfection of the activated c-Ha-ras gene into NIH 3T3 cells makes these cells simultaneously tumorigenic (9–11, 17, 27), invasive in vitro (11, 13), and metastatic in outbred nude mice (9–11, 27). The ability of ras-transfected NIH 3T3 cells to metastasize in immunocompetent mice seems to be influenced by another yet unidentified gene (9) which is possibly involved in the cellular resistance to immunorejection. Recently, reports appeared showing that the ras oncogene is also able to transform rat embryo cells to tumorigenic, invasive, and metastatic cells in syngeneic rats or nude mice (28, 29).

It is noteworthy that the organ distribution of metastases exhibited by ras-transfected lymphoma cells was comparable to that of T-cell hybrids (14, 15) and also of many spontaneous lymphoma cells (30, 31). For instance, the liver was the major affected organ, whereas no metastases were found in the lungs even though this is the first organ encountered by tail vein injected cells. In contrast, i.v.-injected ras-transfected mouse and rat fibroblasts and mammary carcinoma cells form predominantly lung metastases. This suggests that the ras gene product turns on a metastatic program specific for the cell type in which it is transfected, a conclusion also drawn from recent transfection studies using various murine recipient cells that showed different metastatic potential (27).

Comparison of ras gene expression of three transficients and the cells from the corresponding metastases revealed a variation in ras specific transcription. For instance, the metastases derived from BW(neoEJ)11 exhibited a considerably decreased level of ras expression. Comparable results have also been reported for ras-transfected mammary carcinoma cells (26). In that study a metastasis was isolated of which the cells had lost the expression of the ras gene. The transcription of the transfected gene is apparently variable while it is still integrated in the host DNA. These data suggest that, once a tumor cell has invaded and metastasized, a high expression of the gene is not necessarily needed for the independent growth at the metastatic site. In this concept, metastases can be isolated exhibiting a lower level of ras gene expression than the corresponding transficients. Maybe this is also an explanation for the contradictory results reported on the relative level of the ras-encoded p21 in benign, primary, and metastatic tumors (6, 7, 32).

The mechanisms underlying the induction of invasiveness and metastatic capacity by the p21 are not clear because the precise role of p21 has not yet been defined. One possible mechanism is the alteration of glycosylation of cell membrane glycoproteins (17, 33), molecules which are thought to play a key role in cell adhesion, invasion, and metastasis (34). The ras protein binds GTP and GDP and, as such, resembles guanine nucleotide binding regulatory proteins (35). In addition, p21 is bound to the inner surface of the plasma membrane (36) and may be part of a signal-transducing complex similar to the membrane bound guanine nucleotide components of the adenylate cyclase system (37). The possible role of p21 in a more general signal transmission pathway which may be involved in activation of different properties in various cell types fits in with the observations that the type of invasiveness and the metastatic pattern induced by p21 are cell type specific. By analogy, it may be suggested that gene products which induce invasiveness in normal T-cells and T-cell hybrids are involved in similar membrane signalling systems.

ACKNOWLEDGMENTS

We thank Dr. C. Gorman for providing the pRSVneo plasmid. Thanks are also due to E. Kamst, I. V. van de Pavert, and K. Gillis for excellent technical assistance and T. Eggenhuisen for help in preparation of the manuscript.

REFERENCES

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