The Beige/Chediak-Higashi Syndrome Gene Encodes a Widely Expressed Cytosolic Protein*

(Received for publication, September 3, 1997)

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The human autosomal recessive disorder Chediak-Higashi syndrome and its murine homologue beige are associated with the formation of giant lysosomes that cluster near the perinuclear region of cells. We prepared a polyclonal antiserum against a glutathione S-transferase-Beige fusion protein and demonstrated by Western analysis that the beige gene encodes a protein of 400 kDa that is expressed in cultured murine fibroblasts as well as most mouse tissues. The protein was not detected in either cultured fibroblasts or mouse tissues from two different beige mutants. Cultured fibroblasts transformed with multiple copies of yeast artificial chromosomes that contain the full-length beige gene showed much higher levels of Beige protein than either wild type fibroblasts or mouse tissues. Subcellular fractionation experiments demonstrated that the Beige protein was cytosolic and, under the conditions of isolation, had no measurable membrane association. Cultured mouse fibroblasts in which the Beige protein was overexpressed had smaller than normal lysosomes that were more peripherally distributed than in control cells. These findings, coupled with earlier published results, suggest that the Beige protein regulates lysosomal fission.

Chediak-Higashi syndrome (CHS) is characterized clinically by partial ocular and cutaneous albinism, susceptibility to pyogenic infections and abnormal platelet function (1). The disorder is lethal and generally ends in an accelerated phase characterized by infiltration of tissues by mononuclear cells. CHS is transmitted as an autosomal recessive trait with similar disorders described in mice, mink, cattle, cats, and killer whales. The best studied of the animal disorders is the murine homologue of CHS designated beige.

Cells from patients with CHS and from beige mice contain giant intracellular vesicles that cluster around the nucleus. Affected vesicles include lysosomes, melanosomes, platelet dense granules, and cytolytic granules. It is not known whether giant vesicles form because of a defect in vesicle fusion or fission (2, 3). The genes responsible for CHS and beige have been identified and are orthologous (4–6), with the predicted sequence of the Beige/CHS protein yielding little insight into the protein’s function.

We demonstrate here that the murine Beige protein is a cytosolic protein expressed in most tissues. A deficiency of Beige protein results in a perinuclear clustering of giant lysosomes. In contrast, overexpression of Beige protein results in abnormally small lysosomes, which localize to the periphery of cells. We suggest that the Beige protein is involved in regulating vesicle fission and not fusion.

MATERIALS AND METHODS

Generation of Polyclonal Antibodies against the Murine Beige Protein—The pGEX-2T (Pharmacia Biotech Inc.) vector was used to make a recombinant fusion protein that contained glutathione S-transferase (GST) linked to the carboxyl-terminal 89 kDa of the murine Beige protein (GenBank accession number U52461). This portion of the Beige protein contains the highly conserved BEACH domain and seven WD40 repeats (4, 5). This construct was made using PCR to synthesize the beige gene portion using the F primer 5’-ACAGGATCCCGTGCAAGTGAATCCATCAGA-3’ and the R primer 5’-AGCGAATTCTCATCCAGCTGCGTAGCTGCT-3’ which contained a BamHI site at its 5’ end and a R primer 5’-ACGGAATTCCTCCTACATCCGTGCTAGTGC-3’ that contained a BamHI site at its 5’ end. These two primers were used with the high fidelity PCR polymerase Expand Long Template PCR System (Boehringer Mannheim) and a template cDNA plasmid named 22B (4), which contains the last 5.6 kb of the murine beige gene. This mixture was amplified using a Perkin-Elmer PCR machine and the following PCR parameters: 94°C for 2 min hot start, followed by 94°C for 30 s, 53°C for 30 s, and 72°C for 4 min for 35 cycles. This reaction yielded the expected band of 2.37 kb, which was then cut with EcoRI and BamHI and gel purified. This EcoRI-BamHI fragment was cloned into the EcoRI-BamHI site of pGE2-2T using standard cloning techniques.

Recombinant plasmids were recovered and assayed for production of the GST-Beige fusion protein (BP55). One clone was used for all of the large scale preparations of the GST-Beige fusion protein as per the manufacturer’s instructions. The glutathione-Sepharose purified fusion protein was digested with thrombin to separate GST from the Beige protein portion. This digested protein mixture was run out on a 6% SDS-PAGE gel, stained with 0.05% Coomassie Brilliant Blue-R250 in distilled H2O, and the 89-kDa recombinant Beige protein band excised. Some of this band was transferred to PVDF membranes, and the amino terminus was sequenced. The sequence obtained precisely matched the expected Beige protein sequence. This gel purified band was mixed 1:1 with complete Freund’s adjuvant and injected into New Zealand White rabbits. All following immunizations were similar except that incomplete Freund’s adjuvant was used.

After five boosts, immune serum was obtained from one rabbit that recognized the starting Beige antigen as determined by Western blotting. This serum was further purified by column chromatography in which the antigen was coupled to Sepharose beads using the manufacturers instructions (Pierce).
Preparation of Fibroblast and Mouse Tissue Extracts, Subcellular Fractionation, and Western Blotting—Mouse fibroblasts (described in Refs. 4 and 7) were maintained in Dulbecco's modified Eagle's medium plus 10% fetal bovine serum or in some instances 1% fetal bovine serum with penicillin and streptomycin (P/S). All cells used in this study, except the Ln10.1 cells, were obtained from the American Type Culture Collection (ATCC). The Ln10.1 cells were kindly provided by Dr. G. N. Martz. When confluent, the monolayers were washed twice with phosphate-buffered saline (PBS) and trypsinized, centrifuged at 200 × g for 5 min, and resuspended in ice cold PBS buffer (250 mM sucrose, 20 mM HEPES, pH 7.2, 50 mM KCl, 1 mM MgCl₂, 0.5 mM EGTA, 20 mM leupeptin (Sigma), 10 mM pepstatin A (Sigma), and 1 mM [4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride] (ICN)). Some experiments were done in a FB buffer that lacked sucrose but that contained 0.5% Triton X-100. Cells were placed in a Donnue homogenizer and centrifuged at 400 × g for 5 min to obtain a postnuclear supernatant (PNS). Mouse tissue homogenates were prepared as described above. Protein determinations were performed using the BCA method (Pierce) and bovine serum albumin fraction V (Sigma) as a standard.

Postnuclear supernatants were fractionated over 10–60% (w/w) linear sucrose gradients for 3 h at 100,000 × g. Fractions were collected from the bottom of the gradient and pelleted at 200,000 × g for 90 min. The pellets were resuspended in 100 μl of a 1% Triton X-100, phosphate-buffered saline, which contained the previously mentioned protease inhibitors, and then analyzed using SDS-PAGE and Western blotting. Hexoseaminidase activity was assayed as described previously (9), and refractive indices were determined on each gradient. All experiments were performed at least three times.

Precast 4–20% gradient gels (Bio-Rad) or 5% SDS-PAGE gels that did not have a "sticker" were used to separate the protein extracts described above. The SDS-PAGE gels were transferred to PVDF membranes (Gelman Sciences) for 1–2 h using electrophoretic transfer described above. The SDS-PAGE gels were transferred to PVDF membranes (9), and refractive indices were determined on each gradient. All experiments were performed at least three times.

**RESULTS**

**Analysis of Lysosome Size by Flow Cytometry—** Cultured fibroblasts were incubated in 1 mg/ml FITC-dextran (10,000 molecular weight, Molecular Probes) in the standard culture medium described above for 18 h. Cells were washed twice with culture medium and then cultured 2 h in culture medium lacking the fluorescent ligand. Culture medium was then replaced with 4% Hanks’ minimal essential medium, and the samples were kept on ice throughout the remainder of the experiment. The cells were scraped and washed once in FB buffer followed by Donnue homogenization in 0.5 ml of FB plus protease inhibitors. Homogenates were centrifuged at 400 × g for 5 min, and the PNS was collected and mixed with 2.5 ml of FACs loading buffer (257 mM sucrose, 20 mM HEPES, pH 7.2, 1 mM EGTA). Flow cytometric analysis was performed on a Becton Dickinson FACS Vantage operating with CellQuest software and equipped with a Coherent Enterprise argon laser (Coherent Laser Group) producing 225 mW at 488 nm. For this application the basic instrument configuration was altered by removing the beam expanding lens in the laser focusing optics, removing the ND1 filter in front of the forward light scatter (FSC) detector and setting the acquisition threshold at 150 on log side scatter (log SSC). All other optical filters for light scatter and fluorescence remained in the basic configuration recommended by the manufacturer for FITC fluorescence. Optical alignment was optimized using DNA QC 2-μm beads (Becton Dickinson) for log FSC, log SSC and log FL1 (FITC) fluorescence. Compensation of the alignment process was confirmed on a daily basis in two ways: For light scatter, a panel of four polystyrene beads ranging in size from 0.48 to 1.43 μm (Polysciences, Inc.) was analyzed, and peak channels were compared with previous results. For log FSC the largest bead appeared just above the 10^4 division on a four log scale (linear channels 1200–1300) and the smallest beads at or below the 10^4 division (linear channels 5–20). The log SSC peaks ranged from the 10^2 (linear channels 70–130) down to 10^2 (linear channels 5–20). In a similar manner, FITC sensitivity was standardized with a FITC Quantum fluorescence kit (Flow Cytometry Standards) that consisted of a blank bead and four beads ranging in fluorescent intensity from 10,000–256,000 mean equivalent fluorescent units.

Before data acquisition, sample buffer was analyzed to assess the noise level due to scattered laser light, electronic noise, and particles in the buffer. For a 2-min data acquisition period, the noise level typically ranged from 1000 to 5000 events. All background events were nonfluorescent (i.e., they were relatively low in the first decade of the FL1 distribution), so a data acquisition gate was set to include only those events positive for FITC fluorescence (>10^3 for log FL1). Data on 50,000 fluorescent events were collected and stored in list mode files for off-line analysis using CelicQuest software (Becton-Dickenson). Lysosome size was measured as forward scatter height (FSC), and different samples were compared by computing the mean and median forward scatter heights on all 50,000 fluorescent events. The data presented in Fig. 3 are representative of four different experiments, all of which gave similar results.

**Visualization of Fibroblast Lysosomes and Immunofluorescence—** The visualization of mouse fibroblast lysosomes was performed as described previously in Refs. 11 and 12. Briefly, fibroblasts on coverslips were incubated in the above mentioned culture medium that contained either 0.5 mg/ml Lucifer Yellow-CH (Aldrich) or 1 mg/ml FITC-dextran (Molecular Probes) for 18 h. The medium was removed, and the cells were washed with fresh medium without the fluorescent probe, followed by a chase in culture medium for at least 2 h. The cells were visualized and photographed using fluorescent microscopy as described in Ref. 12.

**RESULTS**

**Western Analysis of the Beige Protein—** Western blot analysis of mouse fibroblasts, YAC-complemented bg/bg Clones, and mouse tissues. A. Western analysis of mouse fibroblasts. 25 μg of mouse fibroblast protein extracts were run on 5% SDS-PAGE gels and transferred to PVDF membranes for Western analysis using the affinity purified anti-Beige antibodies. B, 50 μg of mouse tissues and the YAC-complemented clone 195-3 were run on 5% SDS-PAGE gels and analyzed as described for B. WT, wild type; kd, kDa. C, antigen blocking experiments. 20 μg of protein from cell extracts from the YAC-complemented clone 195-3 (lane 1) or 50 μg of protein from mouse lung extracts (lane 2) were run on 4–20% SDS-PAGE gel and transferred to PVDF membranes for Western analysis using either anti-Beige antibodies that had been preincubated with purified GST or with the GST-Beige fusion protein antigen.
of the large band is similar to the Beige protein’s predicted molecular mass of 425 kDa (13). These bands, which were unaffected by preincubation of the antibodies with GST, were not observed when the antibodies were preincubated with the intact GST-Beige fusion protein (Fig. 1). The smaller band may represent the heterotrimeric G-protein β subunit, because an identically sized band was recognized by a commercially available antiserum raised against a carboxyl-terminal peptide of the mouse G-protein β subunit (data not shown). Cross-reactivity between these two proteins might be expected due to the presence of conserved WD40 repeats in the G-protein β subunit (14) and in the Beige protein (4).

The cloning of the murine beige gene was confirmed by the identification of a unique mutation within the bg allele (15), which is predicted to result in a truncated Beige protein that is missing the region of Beige that was used to create the GST-Beige fusion antigen (4, 13). Fibroblasts from bg/bg mice lacked the 400-kDa band present in control mouse fibroblasts (Fig. 1B). The bgβ allele has been shown to be associated with reduced levels of beige gene mRNA (6). The 400-kDa band was absent from extracts of bgβ/bgβ mouse fibroblasts (Fig. 1B). In all tissue extracts, both control and mutant, a band larger than 400 kDa was also detected. This band could represent cross-hybridization to the CDC4L/BGL gene product (16), which is a large gene of unknown function that is expressed in a wide variety of cell types and which is highly related (i.e. 50% identical over a 300-amino acid stretch) to the Beige/CHS protein (4).

We cloned the beige gene using a YAC complementation strategy in which YACs were introduced into bgβ/bgβ mouse fibroblasts (7). During the course of these studies we isolated two complemented cell lines (195-3 and 195-4), in which the beige gene was overexpressed as determined by Northern analysis (4). Overexpression in these cells results from the amplification of the YACs as episomal DNA elements (7). Western analysis of extracts from these two cell lines showed a very strong 400-kDa band (Fig. 1B). This band was increased 13-fold in clone 195-3 and 6-fold in clone 195-4 compared with wild type cells. A third complemented cell line (113-1) that contained a different YAC also showed the expected 400-kDa band but at levels that were equal to those of control fibroblasts. Three cell lines (137-1, 151-1, and 151-2) that contained noncomplementing YACs did not show the 400-kDa band but still showed the unknown larger band (Fig. 1B). These results indicate that the 400-kDa band is the Beige protein due to its presence in all YAC-complemented cell lines, its absence in all noncomplemented cell lines, and its absence in two beige mutants.

Cytosolic Localization of the Beige Protein—Although the beige gene mRNA is expressed at very low levels in vivo, it can be detected by reverse transcription-PCR in most tissues tested (4). The 400-kDa Beige protein was detected in almost all mouse tissues tested, with the highest levels of expression seen in brain, spleen, and lung (Fig. 1C), and undetectable levels seen in heart and small intestine. Most tissues examined showed expression levels similar to that seen in the control mouse fibroblasts. The 400-kDa band seen in control mouse tissues was absent in all bg/bg mouse tissues.

Subcellular fractionation of homogenates from YAC-complemented cell lines or wild type mouse lung indicated that the Beige protein was cytosolic and was not associated with membranes. Subcellular fractionation using sucrose or Percoll gradients indicated that the Beige protein showed no association with lysosomes or any other definable organelle. Using a variety of buffer conditions and nucleotides (i.e. ATP, GTP, or GTPγS), we did not observe any association of the Beige protein with membranes (data not shown).

Examination of the distribution of Beige protein in wild type and uncomplemented cells by indirect immunofluorescence showed a weak diffuse cytoplasmic staining. Examination of the YAC-complemented cell lines also showed a diffuse cytoplasmic staining, but the signal seen was significantly brighter than in wild type or mutant cells (data not shown). The immunofluorescence data are consistent with the biochemical data and indicate that the Beige protein is cytosolic.

Effect of Beige Protein on Lysosome Size and Distribution—We examined the effects of Beige protein overexpression on lysosomal morphology using the fluorescent fluid phase marker Lucifer Yellow to visualize fibroblast lysosomes. All YAC-containing cell lines were derived from a C57BL/6J/bgβ/bgβ mouse fibroblast cell line (MCHSF2) that showed the classic beige/CHS phenotype of a reduced number of giant lysosomes clustered near the nucleus (Fig. 2A and Refs. 4, 7, and 17). The control mouse fibroblast cell line, established from a C57BL/6J mouse, showed the typical wild type morphology of numerous small lysosomes that were scattered throughout the cell but that were predominantly located within the cell body (Fig. 2C). The YAC-complemented clone 113-1, which contained wild type levels of Beige protein, showed a lysosomal
The cytosolic localization of the Beige protein may accurately reflect the distribution of the protein, in which case its role may be to interact with a second protein. This second protein may encode a membrane interaction domain as suggested by the known function of WD40 repeats. An alternative that cannot be eliminated by our data is the lack of membrane association may result from the techniques used for the cell fractionation studies, but no membrane association was observed by immunolocalization.

Overexpression of the Beige protein in YAC-complemented cell lines was the result of a 6–12-fold amplification of the YACs as demonstrated by Southern and fluorescent in situ hybridization studies.

Table I

Lysosome size in mutant and YAC-complemented fibroblast cell lines

| Fibroblast cell lines | Median | Mean |
|-----------------------|--------|------|
| SB/Le (bg/bg)         | 45.32  | 46.1 |
| MCHSF2 (bg'/bg'J)    | 43.71  | 46.83|
| C572CF (wild type)   | 32.78  | 32.03|
| 113-1 (YAC complemented) | 28.42 | 27.47|
| 195–3 (Beige overexpressor) | 22.07 | 23.12|
| 195–4 (Beige overexpressor) | 17.78 | 19.55|

We have begun to define the function of the Beige/CHS protein by developing an affinity purified polyclonal antibody directed against the Beige protein and by observing the effects of overproduction of the Beige protein on lysosome morphology. The specific nature of our antibody preparation was established in five ways. First, a band of approximately 400 kDa was identified on Western blots in multiple mouse tissues and cell lines. The molecular mass of the Beige protein predicted by the gene sequence is 425 kDa. Second, this band was absent on Western blots from bg/bg and bg'/bg' mice. Third, the signal from the 400-kDa band was blocked by the addition of the GST-Beige fusion protein into the Western blotting mix but was not blocked by GST alone. Fourth, the 400-kDa band was present in all YAC-complemented cell lines but was missing in all noncomplemented lines. Finally, the 400-kDa band, seen on Western blots from YAC-complemented cell lines, was identical in size to the band seen in control mouse tissues.

Experiments utilizing subcellular fractionation of fibroblast and tissue extracts localized the Beige protein to the cytoplasm. We were unable to detect any membrane association between the Beige protein and any subcellular fraction. This finding was unexpected for two reasons. First, the Beige protein has homology to the VPS15 and Huntington proteins that encode proteins localizing to both cytosolic and membrane fractions (5). The VPS15 and Huntington proteins may cycle on and off membranes and are believed to be involved in vesicle dynamics. Second, the main morphologic abnormalities in cells lacking Beige/CHS protein occur in membrane bound organelles. In a previous report, we complemented the beige defect in a cell culture system using a somatic cell fusion approach (20). We observed complementation only after a normal and beige lysosome had fused. We concluded that the Beige protein required a membrane interaction to exert its effect, but when the beige/CHS genes were cloned, the predicted protein sequence contained no obvious signal sequence or transmembrane domains. The cytosolic localization of the Beige protein may accurately reflect the distribution of the protein, in which case its role may be to interact with a second protein. This second protein may then interact with membranes.

## DISCUSSION

A histogram of lysosome size is presented for three of the cell types studied in this paper. These results were typical of four independent experiments.

Fig. 3. Flow cytometric analysis of lysosome size. A histogram of lysosome size is presented for three of the cell types studied in this paper.
hybridization analysis (7). Amplification resulted in overproduction of \textit{beige} gene mRNA as determined by Northern analyses (4). Although the \textit{beige} gene cDNA is quite large (11.6 kb of open reading frame), Northern blotting detected only one band in the YAC-complemented cell clones, 195-3 and 195-4 (4). In agreement with the results of our Northern analysis, we detected the 400-kDa band only in YAC-complemented cell lines and wild type murine tissues. The 400-kDa band was also the only band that was increased in intensity in our YAC-complemented cell lines (Fig. 1B). These results strongly suggest that the 400-kDa \textit{Beige} protein band is functionally significant to the \textit{beige} phenotype.

Overexpression of the \textit{Beige} protein in \textit{bg} \textsuperscript{J}/\textit{bg} \textsuperscript{J} fibroblasts produced a phenotype different from that in the \textit{beige} mutants, the wild type cells, or the YAC-complemented clone 113-1, which contained wild type levels of \textit{Beige} protein. Cells lacking the \textit{Beige} protein (most CHS and \textit{beige} alleles are believed to be null alleles; Refs. 4–6) contained large lysosomes clustered near the nucleus. Cells with wild type levels of \textit{Beige} protein contained smaller lysosomes clustered in the cell body. Overexpressing cells contained even smaller lysosomes located near the cell periphery. There is a tight correlation between the observed phenotype and the level of \textit{Beige} protein with highest levels producing a phenotype, exactly opposite to that seen in \textit{beige}/CHS mutants. It remains possible that the complementing YACs overexpress other genes, in addition to \textit{beige}, and that these genes are responsible for the observed phenotype, but this seems extremely unlikely.

We previously demonstrated that \textit{beige}/CHS giant lysosomes are as capable of fusing with other lysosomes as are wild type lysosomes (11, 20). This finding strongly suggests that the formation of \textit{beige}/CHS giant lysosomes results from a reduced rate of fission. The hypothesis that \textit{Beige} protein regulates fission is supported by our finding that cells overexpressing \textit{Beige} protein contain smaller than normal lysosomes. We have recently developed a lysosome-lysosome fusion assay (22) and have determined that cytosol from cells overexpressing the \textit{Beige} protein had no effect on lysosome fusion \textit{in vitro}. This finding, coupled with the smaller than normal lysosomes produced when the \textit{Beige} protein was overexpressed, suggests that the defect in \textit{beige}/CHS is one of lysosomal fission and not fusion. A molecular mechanism mediating fission is not obvious, but it is possible that one domain of the \textit{Beige} protein provides a structural support for lysosomes and that another domain, through a linking protein, pulls at the lysosomal membrane until fission occurs.

\textbf{REFERENCES}

1. Barak, Y., and Nir, E.(1987) \textit{Am. J. Ped. Hem. Oncol.} 9, 42–55
2. Oliver, C., and Essner, E. (1975) \textit{Lab. Invest.} 32, 17–27
3. Spicer, S. S., Sato, A., Vincent, R., Eguchi, M., and Poon, K. C. (1981) \textit{Fed. Proc.} 40, 1451–1455
4. Perou, C. M., Moore, K. J., Nagle, D. L., Misumi, D. J., Woolf, E. A., McGrail, S. H., Holmgren, L., Brody, T. H., Dussault, B. J., Jr., Monroe, C. A., Duyk, G. M., Pryor, R. J., Li, L., Justice, M. J., and Kaplan, J. (1996) \textit{Nat. Genet.} 13, 303–308
5. Nagle, D. J., Karim, M. A., Wolf, E. A., Holmgren, L., Bork, P., Misumi, D., McGrail, S. H., Dussault, B. J., Jr., Perou, C. M., Boissy, R. E., Duyk, G. M., Spritz, R. A., and Moore, K. (1996) \textit{Nat. Genet.} 14, 307–311
6. Barbosa, M. D., Nguyen, Q. A., Tchernev, V. T., Ashley, J. A., Detter, J. C., Blaydes, S. M., Brandt, S. J., Chotai, D., Hodgman, C., Solari, R. C. E., Lovett, M., and Kingsmore, S. F. (1996) \textit{Nature} 382, 262–265
7. Perou, C. M., Justise, M. J., Pryor, R. J., and Kaplan, J. (1996) \textit{Proc. Natl. Acad. Sci. U. S. A.} 93, 5905–5909
8. Srivastava, A. K., and Schlessinger, D. (1991) \textit{Gene (Amst.)} 103, 53–59
9. Edelson, P. J., and Erbs, C. J. (1978) \textit{Exp. Med.} 147, 77–86
10. Deleted in proof
11. Perou, C. M., and Kaplan, J. (1993) \textit{Somatic Cell Mol. Genet.} 19, 459–468
12. McVey Ward, D., Perou, C. M., Lloyd, M. L., and Kaplan, J. (1995) \textit{J. Cell Biol.} 129, 1229–1240
13. Perou, C. M., Pryor, R. J., Naas, T. P., and Kaplan, J. (1997) \textit{Genomics} 42, 336–368
14. Neer, E. J., Schmidt, C. J., Nambudripad, R., and Smith, T. F. (1994) \textit{Nature} 371, 297–300
15. Kelley, E. M. (1957) \textit{Res. News. Mouse News Lett.} 16, 36
16. Feuchter, A. E., Freeman, J. D., and Mager, D. L. (1992) \textit{Genomics} 13, 1237–1246
17. Burkhardt, J. K., Wiebel, F. A., Hester, S., and Argon, Y. J. (1993) \textit{Proc. Natl. Acad. Sci. U. S. A.} 90, 3045–3050
18. Murphy, R. F. (1985) \textit{Proc. Natl. Acad. Sci. U. S. A.} 82, 8525–8528
19. Wilson R. B., and Murphy, R. F. (1989) \textit{Methods Cell Biol.} 31, 293–317
20. Perou, C. M., and Kaplan, J. (1993) \textit{J. Cell Sci.} 106, 99–107
21. Perou, C. M., Perchellet, A., Jago, T., Pryor, R. J., Kaplan, J., and Justice, M. (1997) \textit{Genomics} 39, 136–146
22. McVey-Ward, D., Leslie, J. D., and Kaplan, J. J. (1997) \textit{Cell Biol.}, in press.