Quantitative assay for morphogenesis indicates the role of extracellular matrix components and G proteins

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ABSTRACT A quantitative assay for morphogenesis is described that involves counting the organizing centers (swirling patterns) formed by many cultured fibroblasts. Organizing centers, which are found *in vivo*, represent one of the smallest units of morphogenesis. We show that macroscopically visible organizing centers form by the merger of smaller organizing centers. Parallel orientation of cells on plastic substrata requires cell–cell contact, but organizing centers can develop without cell–cell contact on collagen gels. On collagen gels, the orientation of collagen fibers determines the orientation of cells with respect to one another. Although organizing centers resemble fingerprints, we have shown that a stochastic process determines the spatial orientation of organizing centers. Treatment of transformed cell lines with agents that increase cAMP levels or alter the activity of guanine nucleotide binding proteins resulted in the generation of organizing centers. Cholesterol precursors involved in protein isoprenylation were found to be potent reverse-transformation agents that could alter the two-dimensional morphogenesis of cells. The simple assay described should permit the analysis of morphogenesis at the molecular and cellular levels.

During the last decade, progress has been made in understanding the molecular mechanism of cell adhesion (1, 2). Based on this groundwork, it is now possible to investigate the molecular mechanism that governs morphogenesis. By retroviral insertion mutagenesis and other genetic methods, it has been shown that alterations in collagen, integrins, and cell adhesion proteins result in morphological changes *in vivo* (3, 4). The role of collagen in the tension-driven morphogenesis of tendons (5) and an effect of collagen-fiber orientation on the polarity of cultured cells (6) have been described. Thus, cell-adhesion proteins appear to play decisive roles in morphogenesis at both the cellular and organismic levels.

We have chosen a well-known, but relatively unstudied, morphogenic system for analysis; namely, the multicellular organization of normal fibroblasts into two-dimensional swirls (hereafter referred to as organizing centers) (see Fig. 1). This system has been recognized by cell biologists for many years (7–10) and has been observed *in vivo* (11); however, due to lack of quantitative methods for analyzing morphogenic processes, little is currently known about the molecular or cellular basis of this phenomenon. Thus, we have developed assays for quantitatively measuring parameters governing the morphogenesis of organizing centers. By using these assay methods, we show that extracellular matrix materials, agents affecting cAMP levels, and cholesterol precursors regulate the morphogenesis of organizing centers.

MATERIALS AND METHODS

Cell Culture. IMR-90 karyotypically normal human fibroblasts and Chinese hamster ovary CHO-K1 cells were ob-

tained from, respectively, the Coriell Institute for Medical Research (Camden, NJ) and the American Type Culture Collection and maintained as described (6). IMR-90 cells were used prior to 30 population doublings. Collagen gels were prepared in 25-cm² culture flasks as described (12) by the gelation of a neutralized solution of collagen at 37°C. Cholesterol precursors were obtained from Sigma, and used to treat cells as described (13). Lovastatin was a gift from Merck Sharp & Dohme.

Morphometric Analysis. Cells were monitored with a time-lapse videotape apparatus (model BR-9000U, JVC, Japan) that employed a low-light video camera (model TC2511U8, RCA, Lancaster, PA). Videotape images obtained at $\times 100$ magnification were quite satisfactory for the analysis of cell lengths and orientation. Videotape images were digitized by the JAVA image analysis program (Jandel, Corte Madera, CA) to measure parameters such as cell length, angles between neighboring cells, and areas occupied by cells. At least 50 cells were analyzed for each point and statistical analysis was carried out within JAVA.

An organizing center was defined as a group of four (or more) polarized cells aligned in parallel. For the enumeration of organizing centers, lower ($\times 20$ – $\times 40$) magnification was employed and we found that photographs were more easily analyzed than videotape images. Organizing centers were counted by projecting a 35-mm negative onto white paper. The outlines of the organizing centers were traced to determine both the number and area of organizing centers. The number of organizing centers in at least five microscopic fields was averaged for each point.

RESULTS

Time-Lapse Cinemicrography Studies. Morphogenesis of organizing centers took place in several steps (Figs. 1 and 2). The first sign of organization was noted when cells came within about one cell diameter of one another (Fig. 2A). At this critical density, cells that were nearest neighbors began to align parallel to one another and small organizing centers were formed (Fig. 2B). Subsequently, small organizing centers merged to form increasingly larger organizing centers (Fig. 2C). When the culture had reached confluence, the merger of organizing centers proceeded until macroscopically visible organizing centers appeared (Fig. 2D). Thus, the tendency of cells to align parallel to one another appeared to be the driving force that leads to the assembly of macroscopically visible organizing centers (Figs. 1 and 2).

Although the orientation of small organizing centers was nearly random (Fig. 2A), the large organizing centers formed by the merger process were oriented in a single direction (Fig. 2D). The direction in which an organizing center will eventually align can be predicted at the time of semiconfluence. At semiconfluence, cells in certain areas have attained confluence; it is the angle at which cells in such confluent regions

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Abbreviations: G protein, guanine nucleotide binding protein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A.

FIG. 1. Time-dependent merger of organizing centers. (Upper) Cell culture flasks (25 cm²) were seeded with 2×10^5 IMR-90 human fibroblasts, cultured for the number of days indicated, fixed with neutral buffered formalin, and stained with Wright stain. Large numbers of very small organizing centers were found between days 5 and 8, which subsequently merged to form increasingly larger organizing centers (days 8–18). Photomicrographs of IMR-90 cells at 3 hr (A) and 24 hr (B) are shown: note that the size of organizing center increases with time and that very small organizing centers formed by 3 hr when cells were plated at a density that immediately yields a confluent culture. Photomicrographs of CHO-K1 cells untreated (C) or treated (D) for 2 days with 0.1 mM cholesterol are shown: note that cholesterol induces the formation of organizing centers in a transformed cell line. (Bar = 100 μ m.)

are aligned that determines the angle at which macroscopically visible organizing centers eventually will be oriented (Fig. 2 C and D). Thus, the orientation of organizing centers is due to a founder effect in which the earliest assemblages of cells act like seed crystals in determining the final angle at which surrounding cells will align.

The above qualitative description of the morphogenesis of organizing centers suggests means by which the organization of cells can be quantitatively measured. (i) Multicellular organization begins when semiconfluence is reached. As

indicated below, this milestone can be assessed quantitatively by simply measuring average cell-cell distances vs. time. (ii) The events leading to the formation of macroscopically visible organizing centers can be quantitatively assessed by both counting the number of organizing centers vs. time and measuring their area. As demonstrated below, such quantitative parameters permit assessment of the rate of morphogenesis of organizing centers and determination of the effects of extracellular matrix components and drugs on the development of organizing centers.

FIG. 2. Merger of organizing centers. Videotape images were redrawn with the aid of a graphics program. Time lapse videomicroscopy observations indicate that cells are initially oriented at random on plastic substrata (A). As cell contacts increase due to cell growth and migration, small organizing centers appear (B). At semiconfluence, large organizing centers become apparent (C). After confluence, organizing centers continue to merge to give rise to increasingly larger organizing centers (D). The angle at which an organizing center eventually is oriented is determined by a founder effect that occurs at semiconfluence; i.e., an organizing center that forms during semiconfluence will become the dominant organizing center at full confluence.

FIG. 3. Parameters affecting generation of organizing centers. Cell culture flasks (25 cm²) were inoculated with 5×10^5 IMR-90 cells in 5 ml of culture medium. Cell number was determined daily with the aid of an electronic cell counter (A). Data are the number of cells ($\times 10^3$). Both cell length and the area of organizing centers were determined by image analysis of videotaped cultures; the area of each videotaped field was 0.36 mm². Cells elongated rapidly upon spreading and remained at a fixed length thereafter (B). Data are the length of cells in mm ($\times 10^{-2}$). The area unoccupied by cells was found to decrease rapidly as cells spread and then to decrease to zero as cells became confluent (C). The number of organizing centers was determined by photographing a 4.25-mm² field. The number of organizing centers increased rapidly just after cells attached and then decreased as the merger of organizing centers took place as cells reached confluence (D).

Quantitative Analysis of Morphogenesis. Once cells came into close proximity, large numbers of small organizing centers appeared (Fig. 3D, days 0–2); the number of organizing centers decreased with time as organizing centers merged with one another (Fig. 3D, days 2–8). Both the initial number of organizing centers and the rate of their merger increased as initial cell density increased (Fig. 4). It is noteworthy that when cells were planted at a density comparable to confluence, large numbers of very small organizing centers developed within 3 hr (Fig. 1A).

Effect of Extracellular Matrix on Morphogenesis. The ability of plastic and collagen substrata to support the generation of organizing centers was compared (Fig. 5). Both IMR-90 and CHO cells displayed similar responses to the substrates described below with the following exceptions. (i) IMR-90 cells formed organizing centers more rapidly on plastic substrata than did reverse-transformed CHO cells (compare earliest time points in Figs. 4A and 5A). (ii) The ultimate area of IMR-90 organizing centers was many times larger than CHO cells on plastic (compare latest time points in Figs. 4B and 5C). Although CHO organizing centers formed slowly on plastic substrata (Fig. 5A), very large organizing centers formed within a few hours on collagen gels (Fig. 5B). Organizing centers, >2 cm² and containing $>1 \times 10^6$ cells aligned in parallel, formed within a few hours on 30- μ m-thick collagen gels that were restrained at two or more edges (6). On rigid collagen gels, organizing centers quickly merged to yield organizing centers that occupied an entire microscopic field (Fig. 5B and C); in contrast, on plastic substrata, very small organizing centers persisted even after several days (Fig. 5A and C). On rigid collagen gels (1.1 mg/ml), organizing centers formed and merged rapidly to yield large organizing centers; whereas, on weak collagen gels (0.3 mg/ml), the rate of organizing center formation and merger was decreased (Fig. 5B and C). The decreased ability of weak collagen gels to support the merger of organizing centers may be due to the fragmentation of stress patterns within weak collagen gels.

Effect of cAMP on the Morphogenesis of Organizing Centers. Since *N*⁶,*O*²-dibutyryl adenosine 3',5'-cyclic monophosphate (dibutyryl cAMP) has been shown to induce morphological changes in CHO cells (14, 15), we examined

the effects of several compounds that alter cAMP metabolism. Forskolin and cholera toxin promoted the morphogenesis of organizing centers (Fig. 6). Forskolin binds to and directly activates adenylate cyclase (16), and cholera toxin

FIG. 4. Effect of cell density on the rate of merger of organizing centers. IMR-90 fibroblasts were planted in 25-cm² culture flasks; the cell densities employed were 4×10^6 (●), 2×10^6 (▼), 1×10^6 (■), and 5×10^5 (▲). The number of organizing centers was enumerated (A) and the area of organizing centers was measured (B). The results indicate that the rate at which organizing centers merged (A) and their average area (B) increased in cultures planted at higher cell densities. Data are mean $\pm$ SEM (error bars).

FIG. 5. Effect of extracellular matrix components on morphogenesis. Approximately 2×10^6 CHO-K1 cells were planted in culture medium containing 1 mM sodium butyrate on the following substrates: ○, plastic; △, collagen gels (1.1 mg/ml); □, collagen gels (0.6 mg/ml); ▽, collagen gels (0.3 mg/ml). Although organizing centers take several days to form on plastic substrata (A), very large organizing centers form within a few hours on collagen gels (B). Very small organizing centers formed on plastic substrata compared to those observed on rigid collagen gels (1.1 and 0.6 mg/ml) (C). Numerous smaller organizing centers formed on weak collagen gels (0.3 mg/ml) probably due to the fragmentation of stress patterns within such weak collagen gels.

stimulates adenylate cyclase indirectly by ADP-ribosylation of stimulatory guanine nucleotide binding (G) proteins (17). Pertussis toxin, which activates inhibitory G proteins (17), and Li^+ , which prevents binding of guanine nucleotides to G proteins (18), were both inactive in this system (data not shown). Since it has been demonstrated that cholera toxin treatment increases cAMP levels in CHO cells (19), cAMP appears to be involved in the signaling pathway that controls the morphogenesis of fibroblast organizing centers.

Reverse Transformation Induces the Appearance of Organizing Centers. It has been documented for many years that normal human fibroblasts display swirling behavior (7–9). Puck and coworkers (15, 20) demonstrated that butyrate and dibutyl cAMP can induce testosterone-treated CHO cells to display a morphology characteristic of normal fibroblasts. We found that sodium butyrate and other well-known reverse-transformation agents [dimethyl sulfoxide and dimethyl formamide (21)] also promote the formation of organizing centers in a dose-dependent fashion (Fig. 6).

FIG. 6. Effect of reverse-transformation agents on morphogenesis. Approximately 2×10^6 CHO-K1 cells were planted on plastic substrata in the presence of the drug concentrations indicated. The number of organizing centers was enumerated at day 3. The results indicate that the drugs tested induce morphogenesis in a dose-dependent fashion. ○, Lovastatin; ▲, cholesterol; ●, sodium butyrate; ▼, dimethyl formamide; ■, dimethyl sulfoxide; ▽, forskolin; □, cholera toxin.

*Note that all concentrations are molar except cholera toxin, which is given as mg/ml.

Effect of Cholesterol Precursors on the Morphogenesis of Fibroblast Organizing Centers. In examining the enhancing effect of testosterone on the action of sodium butyrate (14, 15), we confirmed observations that several steroids had effects similar to testosterone (14). This study led to the finding that cholesterol, in the absence of any other agent, promoted the formation of organizing centers (Figs. 1 C and D and 6).

Since the effects of cholesterol on the formation of organizing centers may be due to its ability to feedback inhibit its own biosynthesis (22, 23), we examined the effects of lovastatin, which also prevents cholesterol biosynthesis by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) (13, 24). Lovastatin at 1 μM was found to promote the formation of organizing centers in CHO cells (Fig. 6 and Table 1). Treatment of cells blocked by lovastatin with mevalonic acid, the product of HMG-CoA reductase, reversed the effect of lovastatin (Table 1); however, other cholesterol metabolites tested did not abolish the effect of lovastatin (Table 1).

DISCUSSION

Several lines of evidence indicate that collagen is involved in the parallel alignment of cells. Weiss and Taylor (25) demonstrated that cultured cells would align parallel to the

Table 1. Reversal of lovastatin effects by cholesterol metabolites

Drug treatment	Organizing centers, no. per field
Control	0.8 ± 0.4
Mevalonic acid	0.4 ± 0.0
Squalene	0.4 ± 0.2
Dolichol	2.0 ± 0.7
Isopentyl adenine	0.2 ± 0.2
Cholesterol	38.0 ± 2.2
Lovastatin	32.0 ± 4.1
Lovastatin + mevalonic acid	0.4 ± 0.2
Lovastatin + squalene	31.0 ± 4.0
Lovastatin + dolichol	42.2 ± 3.7
Lovastatin + isopentyladenine	41.4 ± 4.4

Culture medium was supplemented with compounds at the following concentrations: 0.1 mM cholesterol, lovastatin (1 $\mu\text{g}/\text{ml}$), 1 mM mevalonic acid, 0.1 mM squalene, 1 μM isopentyladenine, or 0.1 mM dolichol. Approximately 3×10^5 CHO-K1 cells were planted in 25-cm² dishes in these media, and the number of organizing centers per microscopic field was enumerated at day 2. The results indicate that lovastatin treatment results in the generation of organizing centers. Of the cholesterol derivatives tested, only mevalonic acid reversed the effect of lovastatin. Data are presented as the mean $\pm$ SEM.

bundles of parallel collagen fibers in fish scales. Cells can transmit spatial information by orienting collagen fibers (6). In the present study, we found that very large organizing centers form rapidly on collagen gels even in the absence of cell-cell contact (Fig. 5B). In contrast, cells on plastic substrata required cell-cell contact to form organizing centers that are relatively small and only form after longer periods of time (Fig. 5A). In addition to collagen, other cell-adhesion molecules may also play important roles in the morphogenesis of organizing centers.

The morphology of organizing centers depends on the cell type studied (9). Although some cell lines form swirling patterns, as do IMR-90 and CHO cells studied here, epidermal cells form concentric whorling patterns (9). Hence, the shape of an organizing center is a function of the cell type involved, the nature of its extracellular matrix (Fig. 5), and founder effects that determine the spatial arrangements of organizing centers (Figs. 1 and 2).

Due to the reported effects of sodium butyrate on cell elongation (20), we tested other compounds that induce the differentiation of erythroleukemia cells (26, 27) and found that dimethyl sulfoxide and dimethyl formamide were active in inducing the morphogenesis of organizing centers. Since a large number of compounds have been identified that can induce erythroleukemia cell differentiation (21), we predict that other members of this group of compounds may also induce organizing centers.

Since testosterone had been shown to potentiate the effect of butyrate in inducing organizing centers (14), other steroids were examined. We found that cholesterol alone was capable of inducing the morphogenesis of organizing centers (Table 1 and Fig. 6). Since it has been shown that cholesterol biosynthetic intermediates are required for the activation of the ras protein and other G proteins (28–31), we tested the hypothesis that feedback inhibition of the first enzyme in the biosynthesis of cholesterol (HMG-CoA reductase) by cholesterol (22, 23) may deplete a cholesterol precursor required for maintenance of the transformed state. To test this hypothesis, we employed lovastatin, an inhibitor of HMG-CoA reductase (13, 24), and found that treatment of cells with low concentrations of lovastatin induced the formation of organizing centers (Table 1 and Fig. 6). The effect of lovastatin was abolished by mevalonic acid, the enzymatic product of HMG-CoA reductase, whereas other cholesterol biosynthetic intermediates were inactive (Table 1). These results suggest that an intermediate between mevalonic acid and squalene may be responsible for the lack of organizing centers observed in transformed cell cultures. Since the intermediates between mevalonic acid and squalene are phosphorylated and are not transported across the cell membrane, we were unable to directly test compounds involved in protein isoprenylation such as farnesyl pyrophosphate or geranyl pyrophosphate.

Cholesterol biosynthetic intermediates have been shown to control the progression of cells through the cell cycle (32, 33) and to induce morphological changes (rounding up) in neuroblastoma and 3T3 cells (34, 35). Posttranslational modification of the *ras* gene product by the cholesterol metabolite, farnesyl pyrophosphate, is required for the membrane association and transforming activity of *ras* (29). The effects of cholesterol intermediates on cellular processes (32, 34, 35) and the formation of organizing centers may be due to the isoprenylation of a *ras*-like G protein. It has been shown that *ras*-like genes in yeast are involved in controlling cell morphology by regulating actin orientation within the cell (36). Miranti and Puck (37) demonstrated that reverse transformation induced by cAMP results in the induction of a phosphorylated actin homolog. The observations above sug-

gest that G-protein modulation of actin orientation may control cell morphology and the formation of higher-order structures, such as organizing centers, in mammalian cells.

In conclusion, this report introduces a simple assay system that should permit analysis of morphogenesis at the molecular and cellular levels. Although there are several cell culture systems that undergo morphogenesis *in vitro* (38–44), the quantitative methods described here provide an exceptionally simple model system for the study of morphogenesis.

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1. Ruoslahti, E. & Giancotti, F. G. (1989) *Cancer Cells* 1, 119–126.
2. Ruoslahti, E. (1988) *Annu. Rev. Biochem.* 57, 375–413.
3. Kramer, J. M., Johnson, J. J., Edgar, R. S., Basch, C. & Roberts, S. (1988) *Cell* 55, 555–565.
4. Wilcox, M. & Leptin, M. (1985) *Nature (London)* 316, 351–354.
5. Stopak, D. & Harris, A. K. (1982) *Dev. Biol.* 90, 383–398.
6. Klebe, R. J., Caldwell, H. B. & Milam, S. B. (1989) *Matrix* 9, 451–458.
7. Elsdale, T. (1968) *Exp. Cell Res.* 51, 439–450.
8. Elsdale, T. & Wasoff, F. (1976) *Wilhelm Roux's Arch. Dev. Biol.* 180, 121–147.
9. Green, H. & Thomas, J. (1978) *Science* 200, 1385–1388.
10. Erickson, C. A. (1978) *Exp. Cell Res.* 115, 303–315.
11. Nubler-Jung, K. (1987) *Development* 100, 171–177.
12. Bellows, C. G., Melcher, A. H. & Aubin, J. E. (1981) *J. Cell Sci.* 50, 299–314.
13. Cutts, J. L., Scallen, T. J., Watson, J. & Bankhurst, A. D. (1989) *J. Cell. Physiol.* 139, 550–557.
14. Hsie, A. W., Jones, C. & Puck, T. T. (1971) *Proc. Natl. Acad. Sci. USA* 68, 1648–1652.
15. Nielson, S. E. & Puck, T. T. (1980) *Proc. Natl. Acad. Sci. USA* 77, 985–989.
16. Seamon, K. B., Padgett, W. & Daly, J. W. (1981) *Proc. Natl. Acad. Sci. USA* 78, 3363–3367.
17. Moss, J. & Vaughan, M. (1988) *Adv. Enzymol.* 61, 303–380.
18. Casey, P. J. & Gilman, A. F. (1988) *J. Biol. Chem.* 263, 2577–2580.
19. Evain, D., Gottesman, M., Pastan, I. & Anderson, W. B. (1979) *J. Biol. Chem.* 254, 6931–6937.
20. Storrer, B., Puck, T. T. & Wenger, L. (1978) *J. Cell. Physiol.* 94, 69–76.
21. Reuben, R. C., Rifkind, R. A. & Marks, P. A. (1980) *Biochim. Biophys. Acta* 605, 325–346.
22. Brown, M. S. & Goldstein, J. L. (1976) *Science* 191, 150–154.
23. Rudney, H. & Sexton, R. C. (1990) *Annu. Rev. Nutr.* 6, 245–272.
24. Sviridov, D. D., Safonova, I. G., Pavlov, M. Y., Kosykh, V. A., Podrez, E. A., Antonov, A. S., Fuki, I. V. & Repin, V. S. (1990) *Lipids* 25, 177–179.
25. Weiss, P. & Taylor, A. C. (1956) *Anat. Rec.* 124, 381.
26. Leder, A. & Leder, P. (1975) *Cell* 5, 319–322.
27. Hoosein, N. M., Brattain, D. E., McKnight, M. K. & Brattain, M. G. (1988) *Exp. Cell Res.* 175, 125–135.
28. Schafer, W. R., Kim, R., Sterne, R., Thorner, J., Kim, S. H. & Rine, J. (1989) *Science* 245, 379–385.
29. Jackson, J. H., Cochrane, C. G., Bourne, J. R., Solski, P. A., Buss, J. E. & Der, C. J. (1990) *Proc. Natl. Acad. Sci. USA* 87, 3042–3046.
30. Finegold, A. A., Schafer, W. R., Rine, J., Whiteway, M. & Tamanoi, F. (1990) *Science* 249, 165–169.
31. Maltese, W. A. (1990) *FASEB J.* 4, 3319–3328.
32. Sinensky, M. & Logel, J. (1985) *Proc. Natl. Acad. Sci. USA* 82, 3257–3261.
33. Owens, D., Collins, P., Johnson, A. & Tomkin, G. (1990) *Biochim. Biophys. Acta* 1051, 138–143.
34. Schmidt, R. A., Glomset, J. A., Wight, T. N., Habenicht, A. J. R. & Ross, R. (1982) *J. Cell Biol.* 95, 144–153.
35. Maltese, W. A. & Sheridan, K. M. (1987) *J. Cell. Physiol.* 133, 471–481.
36. Bender, A. & Pringle, J. R. (1989) *Proc. Natl. Acad. Sci. USA* 86, 9976–9980.
37. Miranti, C. & Puck, T. T. (1990) *Somatic Cell Genet.* 16, 67–78.
38. White, T. E. K., di Sant'Agnes, A. & Miller, R. K. (1990) *In Vitro* 26, 636–642.
39. Rodriguez-Boulton, E. & Nelson, W. J. (1989) *Science* 245, 718–724.
40. Tacchetti, C., Quarto, R., Nitsch, L., Hartmann, D. J. & Cancedda, R. (1987) *J. Cell Biol.* 105, 999–1006.
41. Ingber, D. E. & Folkman, J. (1989) *J. Cell Biol.* 109, 317–330.
42. Paradis, K. & Sharp, H. L. (1989) *J. Lab. Clin. Med.* 113, 689–694.
43. Yamada, M., Moritoh, C., Kawaguchi, M. & Okigaki, T. (1989) *Eur. J. Cell Biol.* 49, 252–258.
44. Mochizuki, Y., Furukawa, K., Mitaka, T., Yokoi, T. & Kodama, T. (1988) *Cell Biol. Int. Rep.* 12, 1–7.