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Advances in Brief

Plasmids with a Mammalian Replication Origin and a Matrix Attachment Region Initiate the Event Similar to Gene Amplification[1]

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Abstract

Gene amplification plays a crucial role in the development of many human malignancies. Amplified genes are frequently localized on double minutes (DMs). We show here that plasmids bearing both a mammalian replication origin and a nuclear matrix attachment region were able to integrate into DMs if transfected to cells having DMs (COLO 320DM). Furthermore, these plasmids triggered the events leading to the *de novo* formation of the structure similar to DMs if transfected to the cells without DMs (COLO 320HSR or HeLa). Autonomous replication of these plasmids was suggested to be a prerequisite for these events to occur, because the presence of the origin sequences in the plasmids was required. The presence of matrix attachment region in the plasmids is also required for these events to occur, suggesting that matrix attachment plays an indispensable role in extrachromosomal replication. This model system will allow us to investigate the mechanism of gene amplification as well as to analyze the autonomous replication of the plasmid with mammalian replication origins.

Introduction

Gene amplification is a major mechanism by which tumor cells acquire unrestricted growth or drug resistances (reviewed in Ref. 1). Cytogenetically, amplified genes are most frequently detected on extrachromosomal DMs[3] chromatin *in vivo*, whereas the long-term passage *in vitro* usually lead to the domination of cells having chromosomal HSR. We and others have shown that elimination of the amplified genes on DMs from tumor cells leads to the reversion of their tumor phenotype and cellular differentiation (2–4). Such elimination is mediated by the selective incorporation of DMs into micronuclei that may be expelled from the dividing cell (3, 5, 6). This micronucleation process is tightly related to the intracellular behavior of DMs during the cell cycle (7). DMs are composed of acentric circular DNA of various sizes (8). Despite their acentricity, DMs are stably segregated into daughter cells by their tethering to the mitotic chromosomes (7–9). Importantly, a recent study revealed that several viral nuclear plasmids including bovine papilloma virus (10), EBV (11), Kaposi's sarcoma-associated herpes virus (12), and SV40 (13) also use the similar mechanism during segregation. Interestingly, a recent study showed that a plasmid with EBV replicon was able to integrate into DMs in tumor cells (14). In regard to plasmids carrying the SV40 origin of replication, successful tethering requires that the viral large T antigen be expressed. However, successful segregation can also be achieved when the plasmid-encoded large T gene is substituted by the nuclear MAR derived from the cell (13). MAR is found frequently at sites close to the replication origin in the mammalian genome, and matrix attachment is hypothesized to play a role in genome replication (15). These observations led us to investigate whether plasmids that bear a mammalian replication origin and a MAR might mimic the intracellular behavior of DMs.

Materials and Methods

Plasmids. The construction of plasmids used in this study was summarized in Fig. 1. The plasmids pEPBG (11.0 kbp) and pSFVdhfr (11.0 kbp) were a generous gift from Drs. John Kolman and Geoffrey M. Wahl (The Salk Institute, San Diego, CA). The former plasmid carries the EBV latent origin of replication (OriP) and *EBNA-1*, as well as the fusion gene green fluorescence protein and G-associated polypeptide (*GFP-GAP*). The latter plasmid bears a 4.6-kbp fragment containing the Ori β derived from the region that is 3′-downstream to *DHFR* (16). The pSFV-V plasmid (6.4 kbp), which lacks an origin, was constructed from pSFVdhfr by deleting the entire *DHFR*-derived sequence by *Not*I digestion. The pNeo.Myc-2.4 plasmid (9.0 kbp; Ref 17) was a generous gift from Dr. Michael Leffak (Wright State University, Dayton, OH). It contains a 2.4-kbp *Hin*dIII/*Xho*I fragment from the promoter region of c-*myc*. This plasmid was used to construct pNeo-V, which lacks an origin, by deleting almost all of the sequences derived from c-*myc* by *Not*I/*Hin*dIII double digestion. The pNeo.Myc ΔSV plasmid, which lacks MAR, was constructed by deleting most of the SV40-derived sequence that exhibits MAR activity (18) by *Bam*HI/*Bsm*I double digestion. Intronic enhancer MAR in the *Ig*κ gene was first amplified by PCR using the pG19/45 plasmid, and the pAR1 plasmid was constructed from the amplified product (19). The pNeo.Myc ΔSV AR1 plasmid was then generated by inserting the MAR sequence from pAR1 into pNeo.Myc ΔSV, thus replacing the MAR-like SV40-derived sequence.

Other Methods. The human colorectal COLO 320DM and COLO 320HSR tumor cell lines were obtained and maintained as described (5). The HeLa cell line was obtained from American Type Culture Collection (CCL-2). All of the plasmids were purified using the Qiagen plasmid purification kit (Qiagen Inc., Valencia, CA) and transfected into cells by the GenePorter 2 lipofection kit (Gene Therapy Systems, San Diego, CA). Blasticidine (10 μg/ml; Funakoshi, Tokyo, Japan; for pEPBG, pSFVdhfr, and its derivative) or 500 μg/ml of Neomycin (Life Technologies, Inc., Rockville, MD; for pNeo.Myc-2.4 and its derivatives) were used to select the transformants. A biotin-labeled micronuclei probe that detects the COLO 320 amplicons was prepared according to our published protocol (5). Metaphase spreading, DIG-labeled probe preparation and FISH were performed according to published protocols (20) as was the *in vitro* nuclear matrix binding assay (19).

Results and Discussion

Plasmid with EBV Replicon Integrates to DMs. Before examining mammalian replicons, we examined the best-characterized episomal vector, namely that based on the EBV replicon (pEPBG). This plasmid replicates autonomously from its viral *cis*-acting OriP sequence, and its tethering to the chromosomes before segregation requires the expression of viral *EBNA-1* (11). We transfected human COLO 320DM tumor cells bearing multiple DMs with this plasmid

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[3] The abbreviations used are: DM, double minute; HSR, homogeneously staining region; MAR, matrix attachment region; DIG, digoxygenin; FISH, fluorescence *in situ* hybridization; DHFR, dihydrofolate reductase.

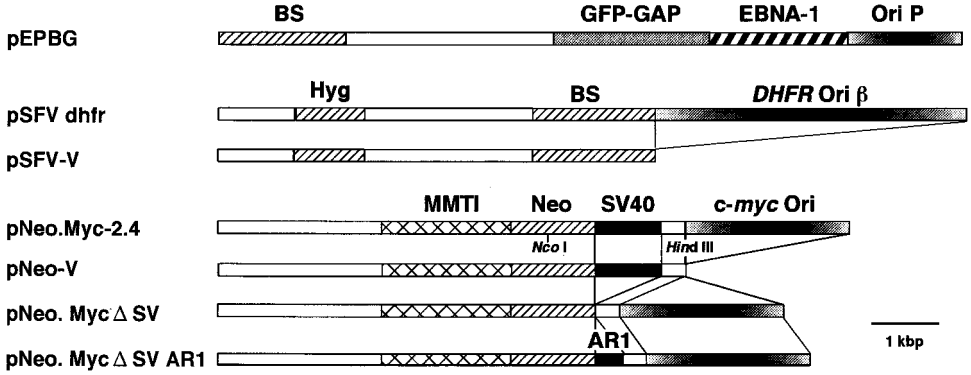

Fig. 1. Plasmids used in this study. pEPBG, pSFVdhfr, and pNeo.Myc-2.4 have EBV replicon (OriP and *EBNA-1*) mammalian origin of replication from *DHFR* locus and c-*myc* locus, respectively. Other plasmids were constructed in this study from pSFVdhfr or pNeo.Myc-2.4. SV40 early region or intronic sequence from *Igκ* gene (*AR1*) exhibit MAR activity and are shown in ■. *DHFR* Ori *β* contained the sequence exhibiting MAR activity inside it (see Fig. 3). *GFP-GAP*, fusion gene between GFP and GAP; *BS*, blasticidin S resistance; *Hyg*, hygromycin resistance; *Neo*, neomycin resistance; *MMTI*, mouse metallothionein 5′ flanking DNA and promoter region. □ represent the vector portion for the growth in bacterial cells.

and first analyzed the mix of stably transformed colonies by FISH. In some metaphase cells, the plasmid sequence was predominantly detected in DMs of various sizes (Fig. 2, *A* and *B*; Table 1). There was no sign of chromosomal integration. These observations are essentially the same as those described in a recent report that showed that a plasmid carrying the EBV replicon selectively recombines with DMs (14). We found that the fraction of cells in the mixed colonies that exhibited plasmid integration into DMs was small (13%), but when clones that expressed high levels of the GFP-GAP fusion protein coded by the plasmid were examined, 10 of 10 clones exhibited DM integration. This suggests that the sequences that become integrated into DMs are expressed at high levels. This is in line with

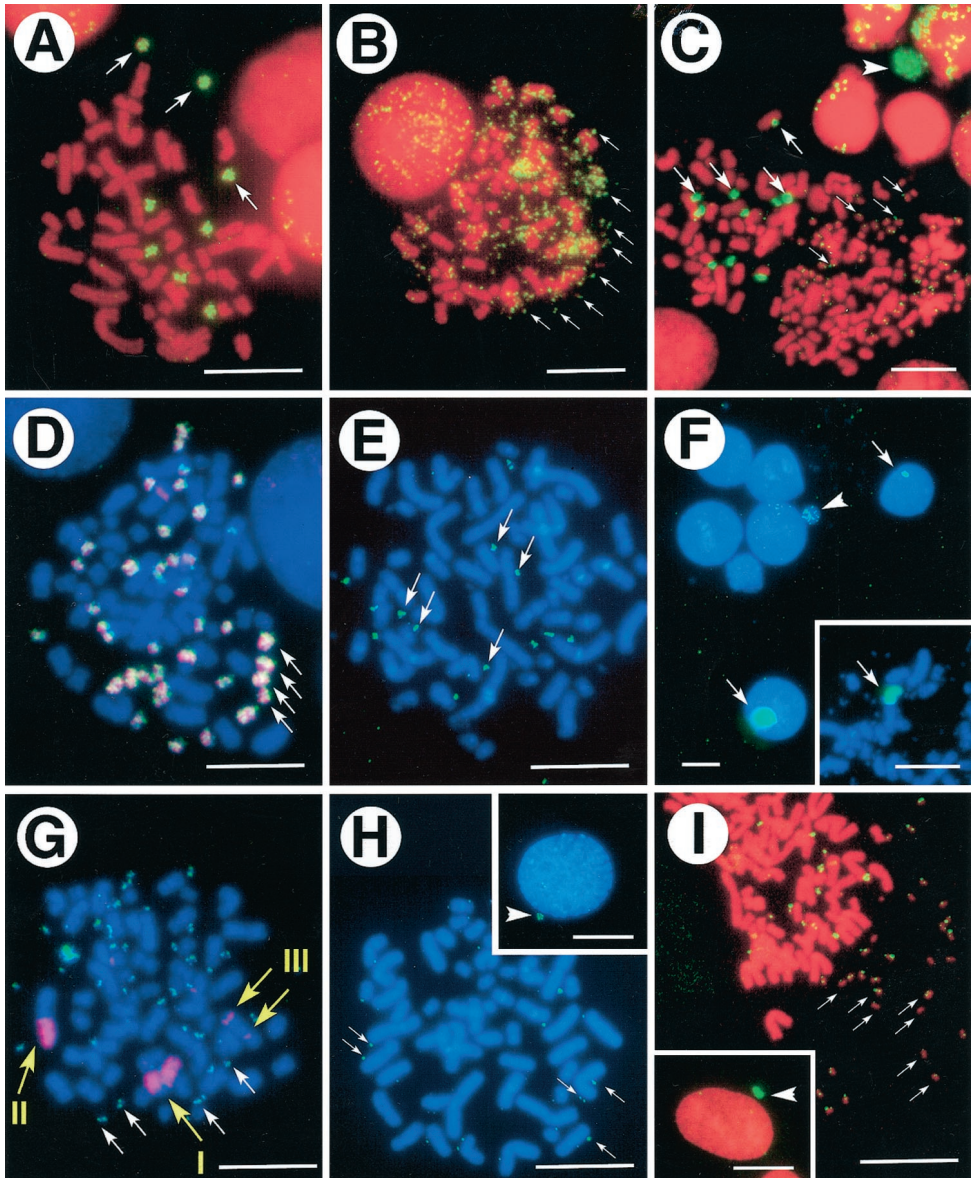

Fig. 2. FISH analysis of tumor cells transformed with plasmids. COLO 320DM (*A–F*), COLO 320HSR (*G* and *H*), and HeLa (*I*) cells were transfected with pEPBG (*A* and *B*), pSFVdhfr (*C, D, G,* and *I*), or pNeo.Myc ΔSV AR1 (*E, F,* and *H*). Stable transformants were selected after >30 days in culture, and metaphase chromosomal spreads were prepared. DIG-labeled probes prepared from the transfecting plasmids were hybridized to the spreads and detected by green fluorescence. For *D* and *G*, a biotin-labeled probe prepared from the purified micronuclei of untransfected COLO 320DM cells was simultaneously hybridized with the DIG-labeled plasmid probe and was detected by red fluorescence. *White arrows* indicate DMs (*A–E* and *G–I*) or giant signals in the interphase nuclei (*F*) that correspond to HSRs in the metaphase (inset of *F*). *Arrowheads* (*F* and *H*) indicate micronuclei enriched with plasmid-derived sequences. DNA was counterstained with propidium iodide as in *red* (*A–C* and *I*), or with 4′,6-diamidino-2-phenylindole as in *blue* (*D–H*). Overlap of *green* and *red* appears as in *yellow*, *green*, and *blue* as in *cyan*, and all three colors as in *white*. In *D*, the plasmid sequence (*green*) coincided with the amplicon sequences (*red*), which is localized on DMs (detected by *blue*, and indicated by *white arrows*). In *G*, the amplicon sequence (*red*) was predominantly detected at the marker HSRs in these cells (*yellow arrow* designated by *I*, *II*, and *III*) but not at DMs (*blue*) with the plasmid sequences (*green* and indicated by *white arrows*). *Bars*, 10 *μ*m.

Table 1 *Frequencies of metaphases showing each type of plasmid localization in the mixture of stable transformants*

Plasmids	Ori	MAR	Signals at DMs[a]	Formation of HSR[b]	Giant signals in interphase[c]
COLO 32ODM					
pEPBG	OriP	OriP	6/45 (13%)	0/45 (0%)	NE[d]
pSFVdhfr	*DHFR*	*DHFR*	28/47 (60%)	5/47 (11%)	NE
pSFV-V	none	none	0/45 (0%)	0/45 (0%)	NE
pNeo.Myc-2.4	c-*myc*	SV40	2/46 (4.3%)	8/46 (17%)	NE
pNeo-V	none	SV40	0/31 (0%)	0/31 (0%)	0/562 (0%)
pNeo.Myc ΔSV	c-*myc*	none	0/22 (0%)	0/22 (0%)	2/314 (0.4%)
pNeo.Myc ΔSV AR1	c-*myc*	*Igκ*	8/25 (32%)	6/25 (24%)	82/553 (15%)
COLO 320HSR					
pSFVdhfr	*DHFR*	*DHFR*	8/40 (20%)	0/26 (0%)	
pSFV-V	none	none	0/45 (0%)	0/45 (0%)	
pNeo.Myc ΔSV	c-*myc*	none	0/32 (0%)	3/512 (0.6%)	
pNeo.Myc ΔSV AR1	c-*myc*	*Igκ*	12/29 (41%)	2/431 (0.5%)	

[a] Frequencies of metaphases showing the plasmid localization at most of DMs among the metaphase.
[b] Frequencies of metaphases showing the formation of chromosomal HSR-like structure composed with plasmid sequences.
[c] Frequencies of interphase nuclei having the giant plasmid signal, which correspond to the HSR-like structure seen in the metaphase.
[d] NE, not examined.

the observations that most of the amplified genes in tumor cells are located on DMs (1).

Plasmids with Mammalian Replicon Also Integrate to DMs. We similarly examined the pSFVdhfr and pNeo.Myc-2.4 plasmids that carry the origin sequences from the *DHFR* (16) and c-*myc* loci (17), respectively (Fig. 1). We found that in COLO 320DM cells these plasmids also become integrated into variously sized DMs and that they are selectively incorporated into the micronuclei (Fig. 2*C*). Simultaneous detection of the plasmid and the amplicon sequences revealed that the DMs with plasmid sequences were derived from the DMs present in untransfected COLO 320DM cells (Fig. 2*D*). The frequency of integration in mixed transformed colonies was high for pSFVdhfr (60%) and low for pNeo.Myc-2.4 (4.3%). It is not clear how the sequence being integrated influences this frequency.

Integration to DMs Requires the Autonomous Replication of the Plasmid. Significantly, if the mammalian origin sequence was deleted from the plasmids (pSFV-V and pNeo-V; Fig. 1), the plasmids did not recombine with DMs (Table 1). We also found that the transformation efficiency was much lower when pSFV-V was used (~30 colonies/10^6 cells) compared with pSFVdhfr (~800 colonies/10^6 cells). These findings suggest that the ability to autonomously replicate is crucial for the integration of the plasmid into DMs and that this seems to increase the transformation efficiency. Why autonomously replicating plasmids frequently recombine with DMs is currently a subject of speculation. One idea is based on the fact that the plasmids and DMs share a common segregation strategy. DMs segregate by tethering to chromosomes after which they tend to be localized at the nuclear periphery at the next G_1 and move to the interior as they are replicated (14, 20, 21). Thus, the possible colocalization of DMs and plasmids in the interphase nucleus may increase the recombination frequency between these two DNA species.

MAR Is Required for the Extrachromosomal Replication Driven by Mammalian Origin. A MAR has been found near the replication initiation sites on the *DHFR* locus (16) but not in the 4.6-kbp insert in pSFVdhfr. Nevertheless, our analysis using the MAR-Finder program (22) predicted the presence of a potential MAR inside this insert, and the predicted fragment was indeed found to bind to the nuclear matrix *in vitro* (Fig. 3). In regard to pNeo.Myc-2.4, the MAR-Finder program did not detect a candidate MAR in the 2.4-kbp insert from the c-*myc* locus. However, the plasmid did contain a sequence from the SV40 early region that had been shown previously to exhibit MAR activity (18). This was confirmed by our *in vitro* matrix-binding assay (Fig. 3). To investigate the role of MAR in transformation and integration into DMs, we deleted the SV40-derived MAR-like sequence in pNeo.Myc-2.4 (thus generating pNeo.Myc ΔSV; Fig. 1), whereas in another construct (pNeo.Myc ΔSV AR1) we replaced it with the MAR from the *Igκ* gene (19). As expected, the *in vitro* binding assay showed that the *Nco*I/*Hin*dIII fragment generated from the former plasmid did not exhibit MAR activity, whereas the one from the latter did bind to the nuclear matrix (Fig. 3). When COLO 320DM cells were transfected with these plasmids, the transformation efficiency was much lower when pNeo.Myc ΔSV was used (~90 colonies/10^6 cells) compared with pNeo.Myc ΔSV AR1 (~800 colonies/10^6 cells). Furthermore, the former plasmid did not integrate into DMs whereas the latter did (Table 1; Fig. 2*E*). These observations indicate that the integration into DMs is dependent on the presence of *cis*-acting MAR. Thus, it appears that the presence of MAR is crucial for the autonomous replication of the plasmid.

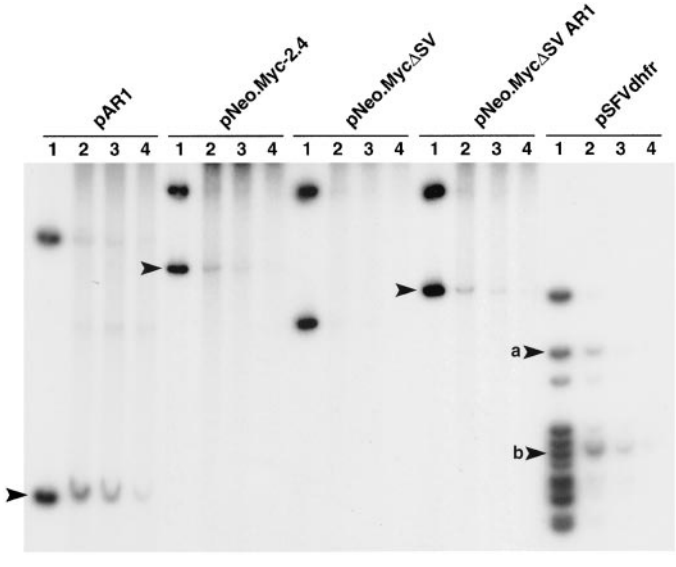

Fig. 3. *In vitro* matrix binding assay. Plasmids were digested either with *Bam*HI/*Hin*dIII (pAR1), *Nco*I/*Hin*dIII (pNeo.Myc-2.4, pNeo.Myc ΔSV, and pNeo.Myc ΔSV AR1; see Fig. 1 for the restriction sites) or *Hin*fI (pSFVdhfr) and then labeled with [α-^{32}P]dATP in an end-filling reaction. The labeled DNA fragments were incubated with nuclear matrix in the presence of increasing concentrations of unlabeled competitor (20:1 mixture of *Escherichia coli* DNA and pUC18 DNA). After washing the matrix by repeated centrifugation, DNA fragments bound to the matrix were separated by 5%-PAGE and visualized by autoradiography. *Lane 1*, input DNA; *Lanes 2–4*, reactions with the competitor DNA (4, 8, and 16 μg, respectively). *Arrowheads* indicate the band specifically bound to the matrix. pAR1 is a control plasmid, which contains a MAR from the mouse *Igκ* gene (indicated by *arrowhead*). The pNeo.Myc-2.4 fragment with MAR activity harbors the SV40 early region that had been demonstrated to be a MAR (18). Deletion of this sequence completely abolished the MAR activity (pNeo.Myc ΔSV). However, insertion of the *Igκ* MAR sequence into this plasmid restored the MAR activity (pNeo.Myc ΔSV AR1). The matrix-bound pSFVdhfr fragment (*a*) was identified as the 886-bp fragment derived from the central portion of the *DHFR* insert that coincided the potential MAR predicted by the MAR-Finder program. Identity of the fragment (*b*) has not been determined, although it is most likely to be an insert-derived fragment as well.

Autonomously Replicating Plasmids Initiate Events Similar to Gene Amplification. When a different cell type was used for the transfection, namely COLO 320HSR cells, an unexpected finding was noted. Although this line does not normally contain DMs, when it was transfected with pSFVdhfr or pNeo.Myc ΔSV AR1, many DMs with plasmid sequences were observed (Fig. 2, *G* and *H*, respectively). Namely, many pairs of minute chromatin were observed among metaphase spread. Two-color FISH revealed that these DMs did not contain the original amplicon sequences seen in COLO 320 cells (Fig. 2*G*), indicating that the DMs were generated *de novo*. DM-bearing cells were frequent only when they had been transfected with plasmids carrying both an origin and a MAR (Table 1). These observations were also noted when HeLa, another tumor line lacking DMs, was used (Fig. 2*I*). The most plausible explanation for this phenomenon is that the plasmids may be recombining frequently with the endogenous extrachromosomal closed circular DNA, which generates large DMs. A broad spectrum of mammalian somatic cells contain extrachromosomal closed circular DNA (23) depending on their genomic plasticity. Our observations directly support the model that suggests that DMs are generated by recombination between microscopically invisible episomes (24).

During these experiments, we found that a proportion of the cells contain very strong plasmid signals along the chromosome arm that resemble the chromosomal HSRs found in tumor cells (Fig. 2*F*, *arrows*). This was observed only when plasmids bearing both an origin and a MAR were used. Interestingly, this frequency was far higher when transfected to COLO 320DM cells compared with COLO 320HSR cells (Table 1). This finding is consistent with the model that proposed that HSRs are formed by the tandem integration of DMs into the chromosomal arm (24).

In summary, in an effort to understand the mechanism of gene amplification, which plays a crucial role in human tumorigenesis, we have developed a new *in vitro* model. This model will also be useful in analyzing the function of mammalian replication origins, because to date most of the work on this subject has used chromosomally integrated sequences that are influenced by the surrounding chromatin. Using our *in vitro* model, we showed here that the integration into or generation of DMs requires a *cis*-acting replication origin and a MAR on the plasmid. This strongly suggests that recombination requires that the plasmid be capable of autonomous replication. Examining this extrachromosomal replication additionally may also facilitate the development of stable plasmids with mammalian replicons that are required by, for example, gene therapy.

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References

1. Benner, S. E., Wahl, G. M., and Von Hoff, D. D. Double minute chromosomes and homogeneously staining regions in tumors taken directly from patients versus in human tumor cell lines. Anti-Cancer Drugs, *2:* 11–25, 1991.
2. Shimizu, N., Nakamura, H., Kadota, T., Kitajima, K., Oda, T., Hirano, T., and Utiyama, H. Loss of amplified c-myc genes in the spontaneously differentiated HL-60 cells. Cancer Res., *54:* 3561–3567, 1994.
3. Von Hoff, D. D., McGill, J. R., Forseth, B. J., Davidson, K. K., Bradley, T. P., Van Devanter, D. R., and Wahl, G. M. Elimination of extrachromosomally amplified MYC genes from human tumor cells reduces their tumorigenicity. Proc. Natl. Acad. Sci. USA, *89:* 8165–8169, 1992.
4. Eckhardt, S. G., Dai, A., Davidson, K. K., Forseth, B. J., Wahl, G. M., and Von Hoff, D. D. Induction of differentiation in HL60 cells by the reduction of extrachromosomally amplified c-*myc*. Proc. Natl. Acad. Sci. USA, *91:* 6674–6678, 1994.
5. Shimizu, N., Kanda, T., and Wahl, G. M. Selective capture of acentric fragments by micronuclei provides a rapid method for purifying extrachromosomally amplified DNA. Nat. Genet., *12:* 65–71, 1996.
6. Shimizu, N., Shimura, T., and Tanaka, T. Selective elimination of acentric double minutes from cancer cells through the extrusion of micronuclei. Mutat. Res., *448:* 81–90, 2000.
7. Tanaka, T., and Shimizu, N. Induced detachment of acentric chromatin from mitotic chromosomes leads to their cytoplasmic localization at G1 and the micronucleation by lamin reorganization at S phase. J. Cell Sci., *113:* 697–707, 2000.
8. Levan, A., and Levan, G. Have double minutes functioning centromeres? Hereditas, *88:* 81–92, 1978.
9. Kanda, T., Sullivan, K. F., and Wahl, G. M. Histone-GFP fusion protein enables sensitive analysis of chromosome dynamics in living mammalian cells. Curr. Biol., *8:* 377–385, 1998.
10. Lehman, C. W., and Botchan, M. R. Segregation of viral plasmids depends on tethering to chromosomes and is regulated by phosphorylation. Proc. Natl. Acad. Sci. USA, *95:* 4338–4343, 1998.
11. Marechal, V., Dehee, A., Chikhi-Brachet, R., Piolot, T., Coppey-Moisan, M., and Nicolas, J. C. Mapping EBNA-1 domains involved in binding to metaphase chromosomes. J. Virol., *73:* 4385–4392, 1999.
12. Ballestas, M. E., Chatis, P. A., and Kaye, K. M. Efficient persistence of extrachromosomal KSHV DNA mediated by latency-associated nuclear antigen. Science (Wash. DC), *284:* 641–644, 1999.
13. Baiker, A., Maercker, C., Piechaczek, C., Schmidt, S. B., Bode, J., Benham, C., and Lipps, H. J. Mitotic stability of an episomal vector containing a human scaffold/matrix-attached region is provided by association with nuclear matrix. Nature Cell Biol., *2:* 182–184, 2000.
14. Kanda, T., Otter, M., and Wahl, G. M. Mitotic segregation of viral and cellular acentric extrachromosomal molecules by chromosome tethering. J. Cell Sci., *114:* 49–58, 2001.
15. Brylawski, B. P., Cohen, S. M., Cordeiro-Stone, M., Schell, M. J., and Kaufman, D. G. On the relationship of matrix association and DNA replication. Crit. Rev. Eukaryot. Gene Expr., *10:* 91–99, 2000.
16. Dijkwel, P. A., and Hamlin, J. L. Matrix attachment regions are positioned near replication initiation sites, genes, and an interamplicon junction in the amplified dihydrofolate reductase domain of Chinese hamster ovary cells. Mol. Cell Biol., *8:* 5398–5409, 1988.
17. McWhinney, C., and Leffak, M. Autonomous replication of a DNA fragment containing the chromosomal replication origin of the human c-*myc* gene. Nucleic Acids Res., *18:* 1233–1242, 1990.
18. Pommier, Y., Cockerill, P. N., Kohn, K. W., and Garrard, W. T. Identification within the simian virus 40 genome of a chromosomal loop attachment site that contains topoisomerase II cleavage sites. J. Virol., *64:* 419–423, 1990.
19. Tsutsui, K., Okada, S., Watarai, S., Seki, S., Yasuda, T., and Shohmori, T. Identification and characterization of a nuclear scaffold protein that binds the matrix attachment region DNA. J. Biol. Chem., *268:* 12886–12894, 1993.
20. Shimizu, N., Itoh, N., Utiyama, H., and Wahl, G. Selective entrapment of extrachromosomally amplified DNA by nuclear budding and micronucleation during S-phase. J. Cell Biol., *140:* 1307–1320, 1998.
21. Itoh, N., and Shimizu, N. DNA replication-dependent intranuclear relocation of double minute chromatin. J. Cell Sci., *111:* 3275–3285, 1998.
22. Kramer, J. A., Singh, G. B., and Krawetz, S. A. Computer-assisted search for sites of nuclear matrix attachment. Genomics, *33:* 305–308, 1996.
23. Wahl, G. M. The importance of circular DNA in mammalian gene amplification. Cancer Res., *49:* 1333–1340, 1989.
24. Gaubatz, J. W. Extrachromosomal circular DNAs and genomic sequence plasticity in eukaryotic cells. Mutat. Res., *237:* 271–292, 1990.

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