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Regulation and mechanisms of gene amplification

KATHLEEN A. SMITH[1], MUNNA L. AGARWAL[2], MICHAIL V. CHERNOV[2], OLGA B. CHERNOVA[2], YUTAKA DEGUCHI[2], YUKIHITO ISHIZAKA[2], THOMAS E. PATTERSON[2], MARIE-FRANCE POUPON[3] AND GEORGE R. STARK[2]

[1] *Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX, U.K.*
[2] *Department of Molecular Biology, Research Institute, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, Ohio 44195, U.S.A.*
[3] *CNRS URA 620, Institut Curie – Section de Biologie, 26, rue d'Ulm, F-75231 Paris, Cedex 05, France*

SUMMARY

Amplification in rodent cells usually involves bridge-breakage-fusion (BBF) cycles initiated either by end-to-end fusion of sister chromatids, or by chromosome breakage. In contrast, in human cells, resistance to the antimetabolite *N*-(phosphonacetyl)-L-aspartate (PALA) can be mediated by several different mechanisms that lead to overexpression of the target enzyme carbamyl-P synthetase, aspartate transcarbamylase, dihydro-orotase (CAD). Mechanisms involving BBF cycles account for only a minority of CAD amplification events in the human fibrosarcoma cell line HT 1080. Here, formation of a 2p isochromosome and overexpression of CAD by other types of amplification events (and even without amplification) are much more prevalent.

Broken DNA is recognized by mammalian cells with intact damage-recognition pathways, as a signal to arrest or to die. Loss of these pathways by, for example, loss of p53 or pRb tumour suppressor function, or by increased expression of *ras* and *myc* oncogenes, causes non-permissive rat and human cells to become permissive both for amplification and for other manifestations of DNA damage. In cells that are already permissive, amplification can be stimulated by overexpressing oncogenes such as c-*myc* or *ras*, or by damaging DNA in a variety of ways. To supplement genetic analysis of amplification in mammalian cells, an amplification selection has been established in *Schizosaccharomyces pombe*. Selection with LiCl yields cells with amplified *sod2* genes in structures related to those observed in mammalian cells. The effect on amplification in *S. pombe* can now be tested for any mutation in a gene involved in repair of damaged DNA or in normal cellular responses to DNA damage.

1. INTRODUCTION

Overexpression, through amplification of oncogenes and of genes that contribute to drug resistance, are important aspects of tumour development (Schwab 1990; Schwab & Amler 1990). Amplified DNA is a prominent manifestation of genetic instability in mammalian cells and is related, in origin, to several other abnormal forms of DNA (deletions, broken chromosomes, etc.) through the common participation of DNA strand breaks (for a review, see Stark *et al.* 1989, 1990; Windle & Wahl 1992; Stark 1993). Formation of broken DNA during the process of amplification stimulates regulation through damage-control mechanisms that recognize and respond to broken DNA in normal mammalian cells and that are defective in most tumours and cell lines. These defence mechanisms lead to the arrest or death of cells in which DNA damage persists and involve important tumour suppressor genes such as p53 and pRb. In this article, we discuss current information on amplification mechanisms in rodent and human cells, especially from the point of view of how the consequences of different mechanisms of amplification may be recognized and dealt with in normal cells.

2. MECHANISMS OF AMPLIFICATION

(*a*) *Rodent cells*

Most work on mechanisms of amplification has been done in cells of Syrian hamster, Chinese hamster and mouse, and has been reviewed recently by one of us (Stark 1993). Briefly, evidence from several laboratories reveals that dicentric chromosomes are a common intermediate and that bridge-breakage-fusion (BBF) is a common process in many – probably most – amplifications in rodent cells. Two BBF cycles are shown in figure 1, where it can be seen that asymmetric breakage of the DNA that lies between the two centromeres of a dicentric chromosome leads to unequal distribution of the intercentromere DNA into the two daughter cells. One daughter receives two copies of some intercentromere markers and the other

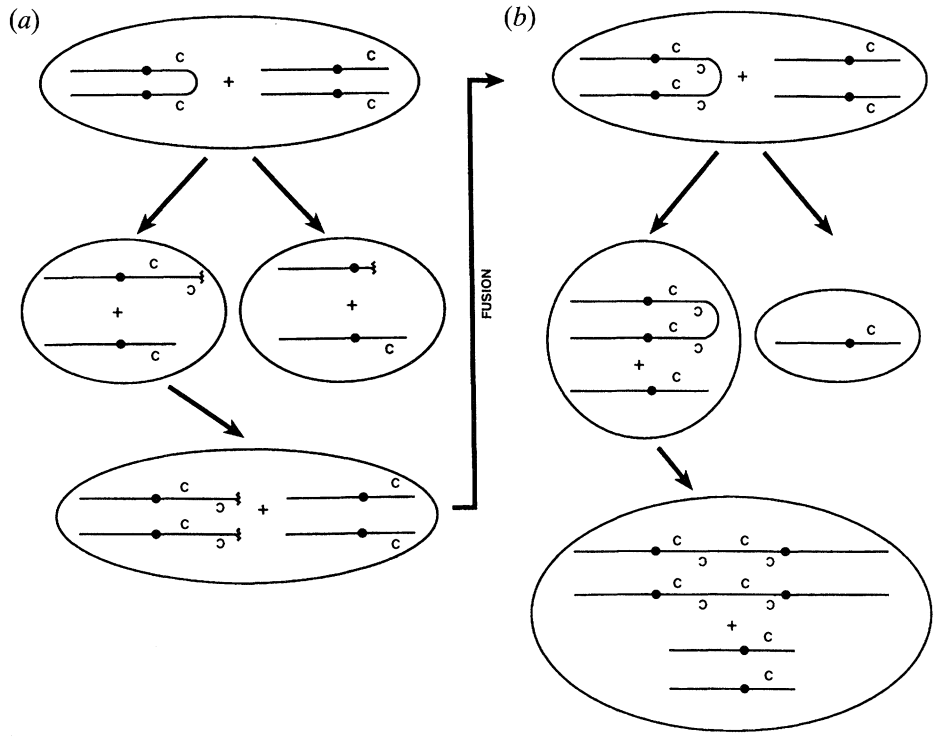

Figure 1. Two bridge-breakage-fusion (BBF) cycles follow the initial formation of a dicentric chromosome, caused by fusing two telomeres of sister chromatids. Note that in the mitotic cell at the top of (*b*), the dicentric chromatid will appear to be a pair of monocentric sister chromatids, whereas after a process such as segregation without breakage (b, bottom), a dicentric sister chromatid (as shown in figure 2) will become apparent. C, a marker gene such as CAD; ●, the centromeres.

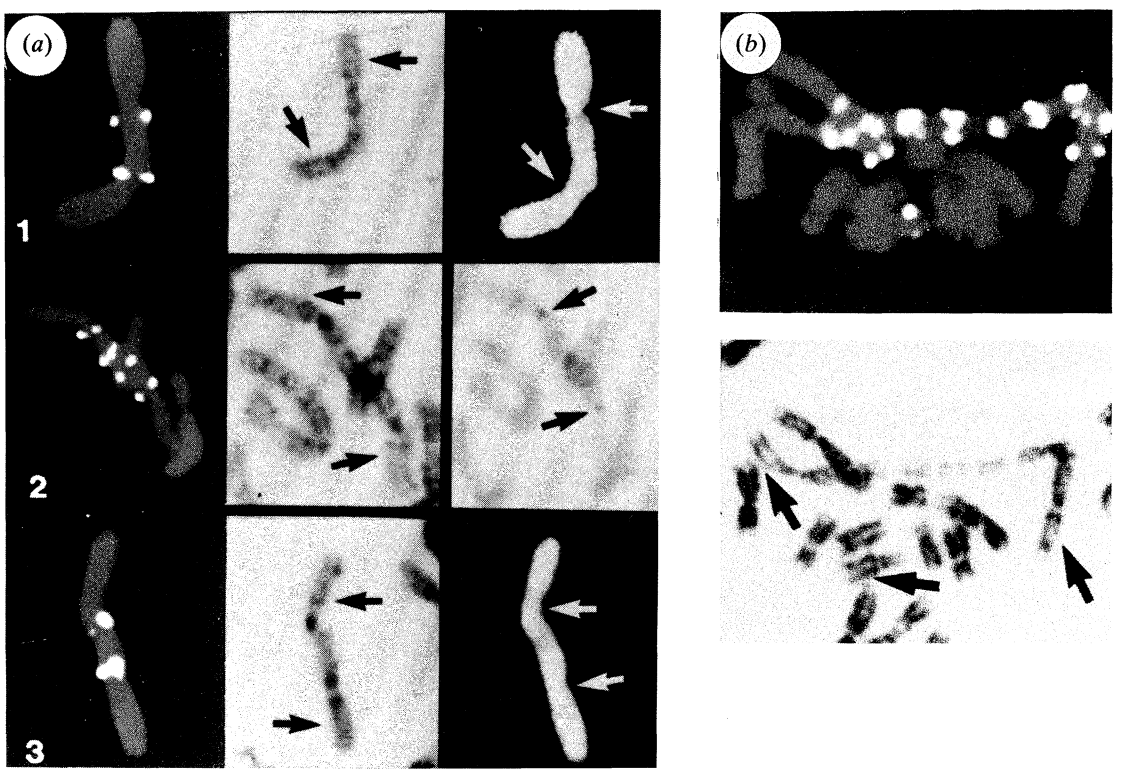

Figure 2. (*a*) Examples of dicentric Syrian hamster chromosomes with amplified CAD genes, present soon after the initial event. The columns (left to right) show *in situ* hybridization, G-banding (arrows point to B9q; the normal location of CAD is on B9p), and for example 2 only, C-banding (arrows point to centromeres). (*b*) A dicentric chromosome containing many amplified CAD genes, present many cell generations after the initial event (arrows point to B9q).

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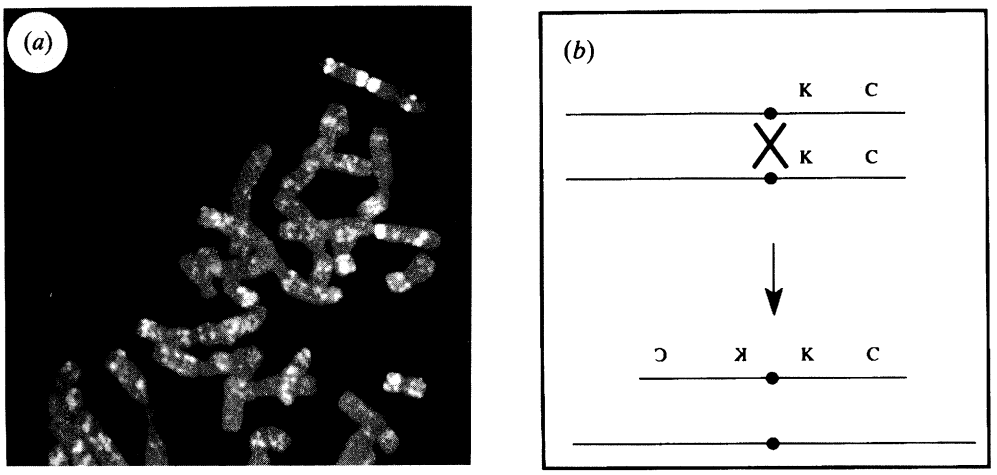

Figure 3. An isochromosome 2p in PALA-resistant HT1080 cells. (*a*) The uppermost chromosome has been derived from human chromosome 2 by centromeric fusion. A normal chromosome 2 can be seen below. The marker chromosome has two p arms with CAD (C) near the telomeres and the V_k gene cluster (K) near the centromere. (*b*) The proposed primary event, involving recombination through the centromeres of sister chromatids. The two centromeres are identical. One has two 'right' portions and the other two 'left' portions. It is important that the recombined centromere on the chromosome bearing CAD retains functions. (Figure and legend reproduced, with permission, from Stark (1993).)

receives none, so that the same basic process leads both to amplification and to deletion. Continuation of BBF events through additional cell cycles can lead to accumulation of multiple copies of a target gene in some of the daughter cells (see figure 1*b*), in arrays that are consistent with the amplified structures seen by *in situ* hybridization analyses of drug-resistant rodent cells (for examples, see figure 2). It is important to note that broken DNA will be present in every daughter cell of a BBF family until some process, such as loss of the broken chromosome before replication, or healing of the broken end through acquisition of telomeric sequences, occurs. Thus any regulatory process that recognizes broken DNA will still be in force many cell generations after initiation of a BBF cycle. It is also important to recognize that BBF cycles can be initiated in two very different ways. (i) fusion first, as illustrated at the top of figure 1*a*. Formation of a dicentric chromosome might result from loss of the repeat sequence TTAGGG from telomeres of cells that lack telomerase activity, as discussed in depth by Stark (1993); or (ii) breakage first (not illustrated). Following replication of a broken chromosome, the adjacent broken ends of two sister chromatids can fuse to form the first dicentric chromosome and initiate BBF cycles.

(*b*) *Human cells*

Relatively little has been done so far to study gene amplification in human cells. Recent work by Schaefer *et al.* (1993) in SV40-infected IMR-90 cells has revealed that, in contrast to rodent cells, human cells can achieve resistance to PALA through formation of an isochromosome 2p and through accumulation of extra copies of chromosome 2. We too have observed an isochromosome 2p in PALA-resistant HT1080 fibrosarcoma cells (see figure 3, K. A. Smith & G. R. Stark, unpublished data, cited in Stark 1993) and have also found additional mechanisms of resistance to PALA in these cells. Thirty-one PALA-resistant HT1080 clones were selected, using a protocol (Smith *et al.* 1990) that insures that the clones result from recent and independent amplification events. Upon analysis by *in situ* hybridization, only two out of 31 clones showed the ladder-like structures commonly seen for amplified CAD genes in Syrian hamster cells (see figure 2*b*) and were presumed to have arisen via BBF cycles. In nine out of 31 cases, isochromosomes 2p were apparent (see figure 3 for an example and explanation). Here, the CAD gene-copy number increases, from the normal two per cell to only three per cell. This modest increase leads to more than a proportional increase in resistance to PALA, accounting for the ability of the cells to survive a selective concentration of drug. In 20 out of 31 cases, analysis by *in situ* hybridization did not reveal any amplified CAD genes. Three of these cases were investigated further: in all three, the specific activity of CAD was about twice as high as in control HT1080 cells, accounting for their resistance to PALA. In two out of three cases, quantitative Southern analysis, using internal normalization with a probe for a single-copy sequence, revealed no significant increase in CAD gene copy number, in agreement with the *in situ* results and suggesting that resistance to PALA was achieved through an increase in CAD transcription or translation, or through stabilization of the CAD mRNA or protein. In the third case, the increase in the specific activity of CAD was accompanied by a comparable increase in gene-copy number. As the extra copies were not seen by *in situ* analysis of fixed metaphase chromosomes in this clone, extrachromosomal amplified DNA may be involved.

In summary, at least five different mechanisms of PALA resistance have been seen in HT1080 and SV40-infected IMR-90 human cells (amplifications as ladders, amplification not as ladders (presumed to be extrachromosomal), isochromosomes 2p, extra chromosomes 2, and increases in the specific activity of CAD without amplification). As discussed next, PALA

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

resistance has not been observed in cells with intact pathways of response to DNA damage, raising the question of how regulation of so many different mechanisms of resistance can be achieved in normal human cells.

3. PERMISSIVITY

(*a*) *Non-permissive cell strains and cell lines*

Observations made in several laboratories show that normal human or rodent cell strains fail to give drug-resistant colonies containing amplified DNA when selected with PALA, methotrexate or hydroxyurea (Lücke-Huhle *et al.* 1987; Lücke-Huhle 1989; Wright *et al.* 1990; Tlsty 1990). Virtually all cell lines do give clones containing amplified DNA, at frequencies typically in the range 10^{-4} to 10^{-6}. A very useful exception is the rat cell line REF52, which does not give colonies containing amplified CAD or dihydrofolate reductase (DHFR) genes upon selection with PALA or metrotrexate (Perry *et al.* 1992*b*). The frequency is now known to be less than 10^{-8} (M. Commane, O. B. Chernova & G. R. Stark, unpublished results). A non-permissive human cell line would be extremely useful for comparative experiments because a much wider range of resistance mechanisms is seen in human cells. Yin *et al.* (1992) worked with non-permissive human cells derived from the Li-Fraumeni line MDAH041 (lacking functional p53) by introduction of wild-type p53 cDNA under control of a constitutive promoter. Unfortunately, these cells, which express p53 in an unregulated manner, have not proved to be stable. We have recently succeeded in preparing a stable non-permissive human cell line from the same MDAH041 parental cells by introducing a construct (kindly provided by Peter Chumakov, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow) in which expression of wild-type human p53 cDNA is regulated by the normal human p53 promoter (Archana Agarwal, M. L. Agarwal & G. R. Stark, unpublished results). The parental permissive cells give PALA-resistant colonies at a frequency of 3×10^{-5}, whereas no colonies were obtained from 6×10^{6} transfected cells. Both selections were done at $3 \times \mathrm{LD}_{50}$ for PALA. These cells can now be used to determine which mechanisms of resistance are observed when the cells are made permissive in different ways.

(*b*) *Involvement of p53, pRb,* ras, *and* myc

The presence of normal p53 in human and mouse cells correlates well with an inability to give rise to colonies with amplified CAD DNA, and loss of p53 from these cells allows them to become permissive for amplification. (Livingstone *et al.* 1992; Yin *et al.* 1992). Both of these groups also found that the presence of wild-type p53 correlated well with the ability to arrest in the G1 phase of the cell cycle in response to the pyrimidine deprivation caused by PALA. These results are readily understood in view of the discussion above, showing that broken DNA often accompanies amplification, and that p53 is a major element in regulating the response of normal cells to DNA damage (for an example, see Nelson & Kastan 1994). The reason for the regulatory effect of p53 on mechanisms of PALA resistance that do *not* involve broken DNA is unclear at present.

Non-permissive REF52 cells have been used to introduce genes that affect permissivity. Not surprisingly, introduction of SV40 large T-antigen, which binds to both p53 and pRb, converts REF52 cells to a permissive state (Perry *et al.* 1992*b*). The effect of T-antigen on REF52 cells results from the loss of p53 because introduction of the dominant negative C141Y mutant of human p53 also permits REF52 cells to achieve resistance to PALA, at a frequency of 7×10^{-5} (O. B. Chernova, M. V. Chernov & G. R. Stark, unpublished results). Conversely, expression of an adenovirus 5 E1A protein, which binds to pRb but not to p53, does not convert REF52 cells to permissivity (Perry *et al.* 1992*b*). Recent work with the E7 oncoprotein of human papillomavirus type 16 (which also binds to pRb) has shown that the ability to arrest growth in G1 in normal human cells in response to DNA damage can be lost in the presence of wild-type p53 (Demers *et al.* 1994; White *et al.* 1994). Although PALA-resistant colonies could be obtained from cells expressing E7, they exhibited polyploidy rather than amplification through rearrangement of DNA (White *et al.* 1994). This result differs from that obtained by Perry *et al.* (1992*b*) with REF52 cells expressing E1A where no PALA-resistant clones were observed.

It is clear that the presence of wild-type p53 is necessary but not sufficient to maintain a non-permissive state. Perry *et al.* (1992*b*) found that, although neither a mutant form of *ras* nor the adenovirus E1A gene alone was capable of converting REF52 cells to permissivity, the combination did so readily, without loss of p53. Similarly, Livingstone *et al.* (1992) found that two cell lines with wild-type p53 were nevertheless permissive.

Overexpression of *myc* genes alone can also convert REF52 cells to permissivity. Introduction of a construct constitutively expressing mouse c-*myc* gives PALA-resistant colonies at a frequency of 5×10^{-5} at $3 \times \mathrm{LD}_{50}$, and introduction of human N-*myc* gives an even higher frequency, 3×10^{-4} (Y. Ishizaka, O. B. Chernova, M. V. Chernov & G. R. Stark, unpublished results). Coamplification of CAD and N-*myc*, which lie very near each other on rat chromosome 6 and human chromosome 2, accounts for the observation that pretreatment of non-permissive REF52 or human MDAH041 (wild-type p53) cells with a low concentration of PALA ($1.5 \times \mathrm{LD}_{50}$) for three days, converts them to a state permissive for CAD gene amplification, whereas pretreatment with a comparable concentration of methotrexate has no effect on permissivity (O. B. Chernova, M. V. Chernov, M. L. Agarwal & G. R. Stark, unpublished results). In an intermediate level of PALA, the cells can divide slowly, for up to ten days, but the stress of replicating DNA under conditions of severe starvation for the deoxypyrimidine triphosphate precursors of DNA may lead to DNA strand breaks and thus to amplification. Since CAD and N-*myc* are near neighbours, they are likely to be

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 1. *Incorporation of BrdU into new PALA^R colonies shifted to a non-permissive temperature*

(Rat REF52 cells expressing the temperature-sensitive SV40 large T-antigen mutant tsA58 were used. Selection at 33 °C for 26 days yielded colonies of 100–300 cells. These colonies were shifted to 39.5 °C for 13 days. The pulse of BrdU was for 3 h.)

PALA^R colonies examined	BrdU$^+$ cells/colony			
	0	1–3	4–10	> 10
22	13	7	0	2

coamplified; increased expression of N-*myc* leads to permissivity and increased expression of CAD to PALA resistance.

(*c*) *Manipulation of permissivity*

The role of p53 in regulating the cellular response to broken DNA present early in an amplification sequence was investigated by using temperature-sensitive (ts) SV40 large T-antigen to inactive p53 in REF 52 cells (Y. Ishizaka & G. R. Stark unpublished results). Although SV40 large T-antigen binds other cellular proteins as well, we know that inactivation of p53 alone is sufficient to make REF52 cells permissive for amplification (see the previous section). The tsA58 mutant was used. At 33 °C, PALA-resistant colonies were readily observed but not at 40 °C, as expected, because T antigen is not present at this temperature. The independent, new colonies that formed at 33 °C were shifted to 40 °C. When 'late' colonies of *ca.* 10^6 cells were shifted, there was no growth arrest and *ca.* 60% of the colonies were labelled with a 3 h pulse of BrdU. Conversely, when 'early' colonies of *ca.* 100 cells were shifted to 40 °C, growth was arrested and only a few cells in under half the colonies were labelled with a pulse of BrdU (see table 1). We interpret these results to indicate that, soon after an amplification event has been initiated, the broken DNA present in most cells triggers growth arrest in the presence of wild-type p53. In time, the broken DNA is lost or the broken ends are healed in most cells; for example, very few dicentric chromosomes carrying amplified CAD genes are seen in PALA-resistant Syrian hamster cells at the 10^6-cell stage (Smith *et al.* 1990). Therefore, despite the fact that the 'late' non-permissive REF52 cells still contain amplified DNA, their growth is no longer regulated by wild-type p53.

4. PROBABILITY OF AMPLIFICATION

(*a*) *Amplificator cells*

The probability of amplification can be increased in permissive cells in a variety of ways. The effects of transient treatment with DNA-damaging agents or metabolic inhibitors are well established and are discussed further below. It is also possible, however, to alter stably the probability of amplification. Selection of permissive cells simultaneously with two drugs gives 'amplificator' clones with rates of amplification that are generally increased, up to 25-fold (Giulotto *et al.* 1987). Furthermore, treatment of permissive cells with 5-aza-2′-deoxycytidine (which leads to extensive and relatively stable demethylation of DNA) increases the rate of amplification substantially (Perry *et al.* 1992*a*). The amplificator phenotype is dominant in cell fusion experiments (Rolfe *et al.* 1988), leading to the hypothesis that overexpression of one or more genes can affect rates of amplification. Two such 'amplificator' genes have already been identified. Denis *et al.* (1991) showed that regulated overexpression of human c-*myc* increased the frequency of DHFR amplification in a permissive rat embryo fibroblast cell line by at least tenfold and similar results have been obtained by Lücke-Huhle (1989) in Chinese hamster cells. More recently, Wani *et al.* (1994) showed that regulated overexpression of a mutant human Ha-ras protein increased the frequency of both DHFR and CAD gene amplification in permissive NIH 3T3 cells, again by a substantial factor. From the above set of observations, it is clear that one may be able to clone 'amplificator' genes by using an expression strategy in which cloned DNA is introduced into a cell line with a low rate of amplification and then recovered from cells that have a high rate of amplification. These experiments are difficult and laborious in practice, as only a small fraction of cells carrying such an amplificator clone will actually amplify a gene such as CAD, allowing their selection. Despite these difficulties, we have isolated a genomic clone from a Syrian hamster amplificator cell line that stimulates amplification of CAD in monkey cells; we are presently characterizing the responsible gene (Y. Deguchi & G. R. Stark, unpublished results).

How might amplificator genes act? Our ideas have expanded with the realization that cells (especially human cells) may achieve drug resistance through a variety of mechanisms and also that permissivity may be enforced by different means, depending on the mechanism of resistance. Clearly, we expect any activity that increases the probability of forming dicentric chromosomes to stimulate amplification. For example, any activity that increases the probability of breaking DNA or of fusing the ends of sister chromatids should increase the rates of amplification through the BBF pathway shown in figure 1. In human cells, we would also expect to increase the rate of CAD amplification by expressing activities that stimulate excision of interstitial DNA. This, in turn, creates extrachromosomal elements capable of autonomous replication that increase either the rate of isochromosome formation through recombination of centromeric DNA, or the rate of chromosome non-disjunction. Finally, if some pathways are permitted in a particular cell line and some are not, an amplificator gene could open one or more forbidden pathways by making the cell tolerant of the resulting genomic change. On the face of it, such activities might be expected of oncogenes such as *myc* and *ras*. Isolation and characterization of new amplificator genes can shed further light on both the mechanisms and the regulation of amplification, possibly with important implications for understanding other manifestations of genomic instability as well.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 2. *Effect of pre-exposure to drugs on rates of amplification in baby hamster kidney (BHK) cells*

pre-treatment (6 days)	selective drug (*ca.* 28 days)	amplification rate ($\times 10^5$)
none	20 μM PALA	7.6
	200 μM PALA	< 0.01
	100 nM methotrexate	3.0
	200 nM methotrexate	0.2
20 μM PALA	200 μM PALA	0.9
	100 nM methotrexate	5.4
	200 nM methotrexate	2.3
100 nM methotrexate	200 μM PALA	0.5

(*b*) *Effects of treatment with DNA damaging agents or metabolic inhibitors*

It has been well appreciated for some time that DNA damage and inhibition of DNA synthesis can transiently increase the rate of amplification in permissive cells (reviewed by Stark & Wahl 1984; Schimke 1988). The effect of DNA damage is readily understood if damage leads to an increased probability of forming a dicentric chromosome or of excising an acentric chromosomal element capable of autonomous replication. Arrest of DNA replication, for example, by starvation for deoxynucleoside triphosphates, can also lead to transient stimulation of amplification. Recent experiments in our laboratory (M.-F. Poupon & G. R. Stark, unpublished results) have shown that pretreating *permissive* baby hamster kidney (BHK) cells with PALA or methotrexate can lead to an increased rate of gene amplification (see table 2). Pretreating BHK cells with 20 μM PALA leads to an increase of about tenfold in the rate at which the cells become resistant to 200 nM methotrexate and, conversely, pretreating the cells with 100 nM methotrexate allows colonies resistant to 200 μM PALA to be observed, whereas no colonies are observed without pretreatment. This result differs markedly from the effect of PALA pretreatment on the ability of the non-permissive REF52 cells to tolerate DNA damage (see above), where there is no effect of methotrexate pretreatment on resistance to PALA, or vice versa, and where coamplification of CAD and N-*myc* is responsible for the effect. In the BHK experiment (see table 2), we propose that starvation for deoxypyrimidine triphosphates (in PALA) or dTTP and deoxypurine triphosphates (in methotrexate) leads to DNA damage, stimulating formation of dicentric chromosomes and possibly other mechanisms of amplification.

5. AMPLIFICATION IN *SCHIZOSACCHAROMYCES POMBE*

We are studying amplification in this fission yeast to take advantage of the fact that genetic and biochemical experimentation can be carried out with relative ease because an extensive collection of mutants are available for use. L-Methionine sulfoximine is used to select for increased expression of *gln1*$^+$, which codes for glutamine synthetase (Barel *et al.* 1988), and LiCl is used to select for increased expression of *sod2*$^+$, which codes for an $Na^+(Li^+)/H^+$ antiporter (Jia *et al.* 1992). As shown in figure 4, our physical mapping of these two genes indicates that they are located on the same arm of chromosome I: *gln1*$^+$ maps to *Not*I fragment D in the middle of the arm; *sod2*$^+$ maps telomere-proximal to *rad8*$^+$ on *Not*I fragment L, within *ca.* 160 kilobases (kb) of the 'left' telomere (Hoheisel *et al.* 1993).

Spontaneous amplification of *sod2*$^+$ is exceedingly rare (*ca.* 2.5×10^{-8}), but can be induced by irradiation with 254 nM ultraviolet (UV) light to 50% survival (T. E. Patterson & G. R. Stark, unpublished results). Under these conditions, the frequency of amplification is *ca.* 3×10^{-7} per cell. The level of amplification is variable; one to ten extra copies have been detected. In two independent strains that have been characterized further, the average amplicon size is 50 to 75 kb. Amplification of *sod2*$^+$ results in at least two structures: in one, the amplified sequences are found on an expanded *Not*I fragment L, in the other, the telomere-containing *Not*I fragment M of chromosome II is also involved (see figure 4). We are currently analysing the amplified DNA at *sod2*$^+$ for the presence of inverted repeats to learn more about the mechanism.

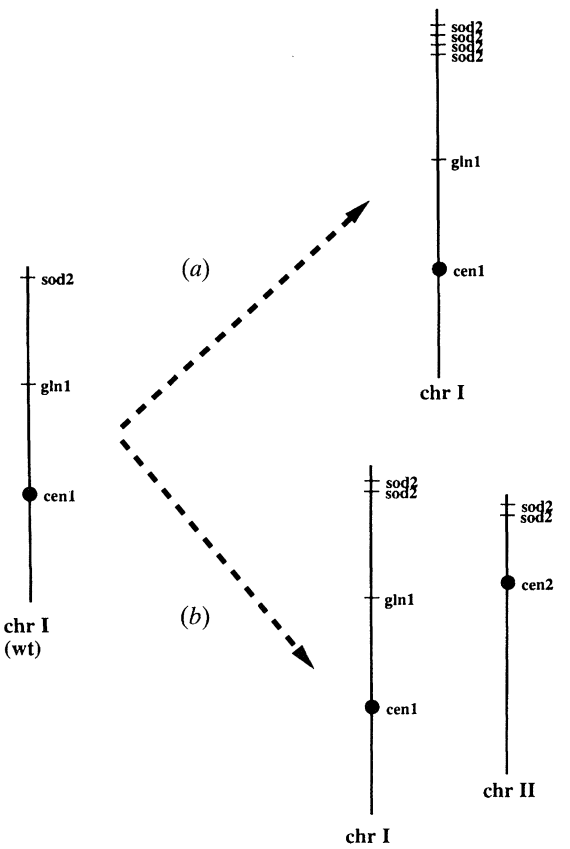

Figure 4. A schematic diagram of gene amplification in *S. pombe*. The two genes studied are located on the same arm of chromosome I, *sod2* near the telomere and *gln1* near the middle of the arm. No amplification of *gln1* has been found. However, *sod2* amplification is found easily. Two cases have been examined in detail. (*a*) the extra copies are found as a tandem array at or near the normal position of *sod2*. (*b*) The extra copies are found near the normal position of *sod2* and also near one telomere of chromosome II.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Although we can detect amplification of *sod2*$^+$, under identical conditions we have not detected amplification of *gln1*$^+$ (frequency less than 2×10^{-9}). Therefore the UV-induced amplification frequency of *gln1*$^+$ is at least 140-fold lower than that of *sod2*$^+$. The organization of these two genes suggests that proximity to a telomere might be an important determinant of amplification in *S. pombe*. To address this issue, we have constructed a strain in which the chromosomal locations of *sod2*$^+$ and *gln1*$^+$ have been exchanged reciprocally, the influence of chromosomal location on the frequency of amplification is now being tested.

We are also taking a genetic approach to understand mechanisms and regulation of gene amplification by screening known mutations in genes that are involved in responses to DNA damage, chromatin metabolism, etc., for their effects on the frequency of gene amplification. Candidates include previously identified *S. pombe* mutations, as well as genes identified in mammalian systems. Preliminary results indicate that a mutation in the cell cycle checkpoint control gene *rad9* (al-Khodairy & Carr 1992), involved in regulating cell cycle arrest in response to DNA damage, leads to a 100-fold increase in the frequency of UV-induced amplification of *sod2*$^+$. This finding reinforces the idea, first generated from studies using mammalian systems, that the ability to progress through the cell cycle in the presence of damaged DNA is linked to the ability to amplify DNA.

6. CONCLUSIONS AND FUTURE DIRECTIONS

Recent work in which gene amplification has been studied in human cells has made it clear that multiple mechanisms are responsible. A major challenge for the future is to determine which specific mechanisms are affected by each treatment or agent that stimulates amplification in permissive cells. Another challenge is to relate the very different consequences of the genomic derangements that accompany different amplification mechanisms to specific regulatory pathways that govern permissivity. It is hard to imagine that the cell responds to abnormalities as different as dicentric chromosomes, isochromosomes or aneuploidy through the same regulatory pathway. Therefore, when a non-permissive cell is rendered permissive by introduction of either c-*myc* on or mutant p53, we do not necessarily expect that the drug-resistant cells will have become permissive in the same way. A detailed investigation of the mechanistic consequences of stimulating or permitting amplification in different ways is likely to yield information of broad interest.

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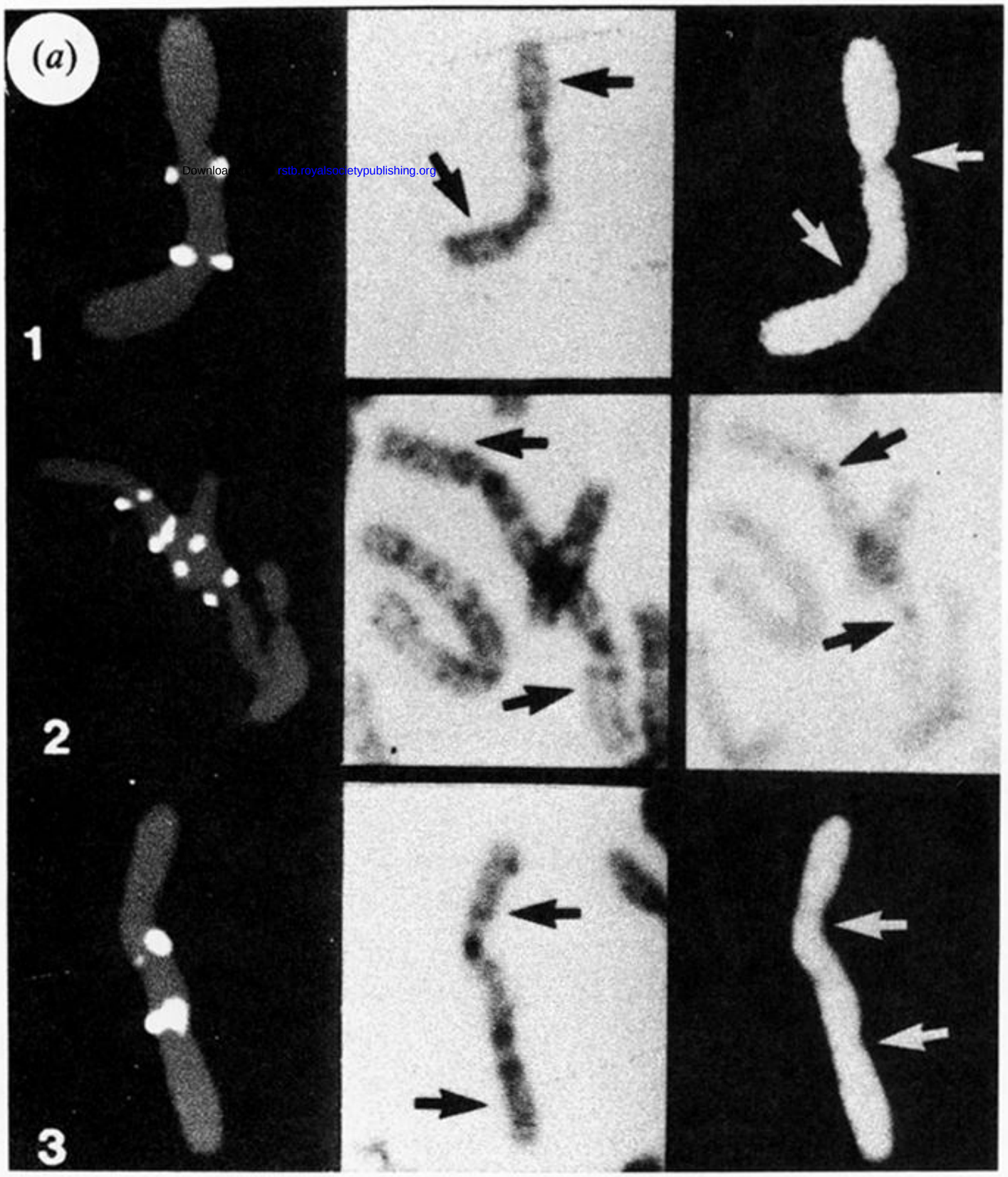

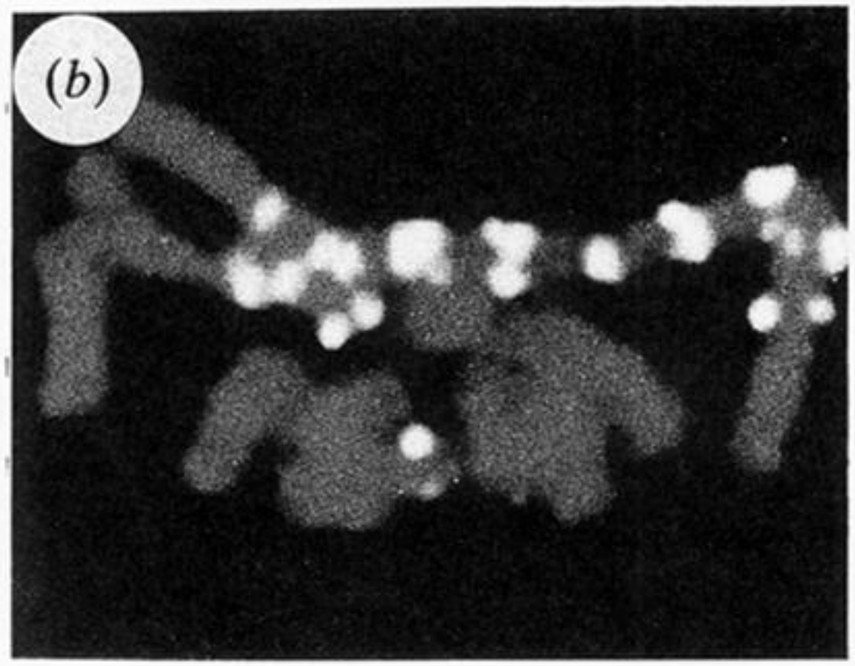

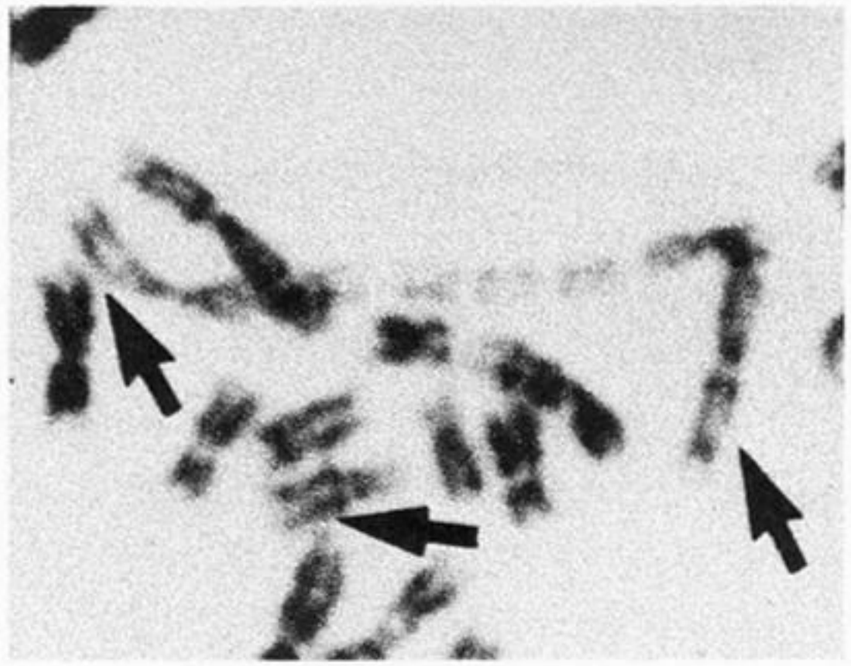

Figure 2. (*a*) Examples of dicentric Syrian hamster chromosomes with amplified CAD genes, present soon after the initial event. The columns (left to right) show *in situ* hybridization, G-banding (arrows point to B9q; the normal location of CAD is on B9p), and for example 2 only, C-banding (arrows point to centromeres). (*b*) A dicentric chromosome containing many amplified CAD genes, present many cell generations after the initial event (arrows point to B9q).

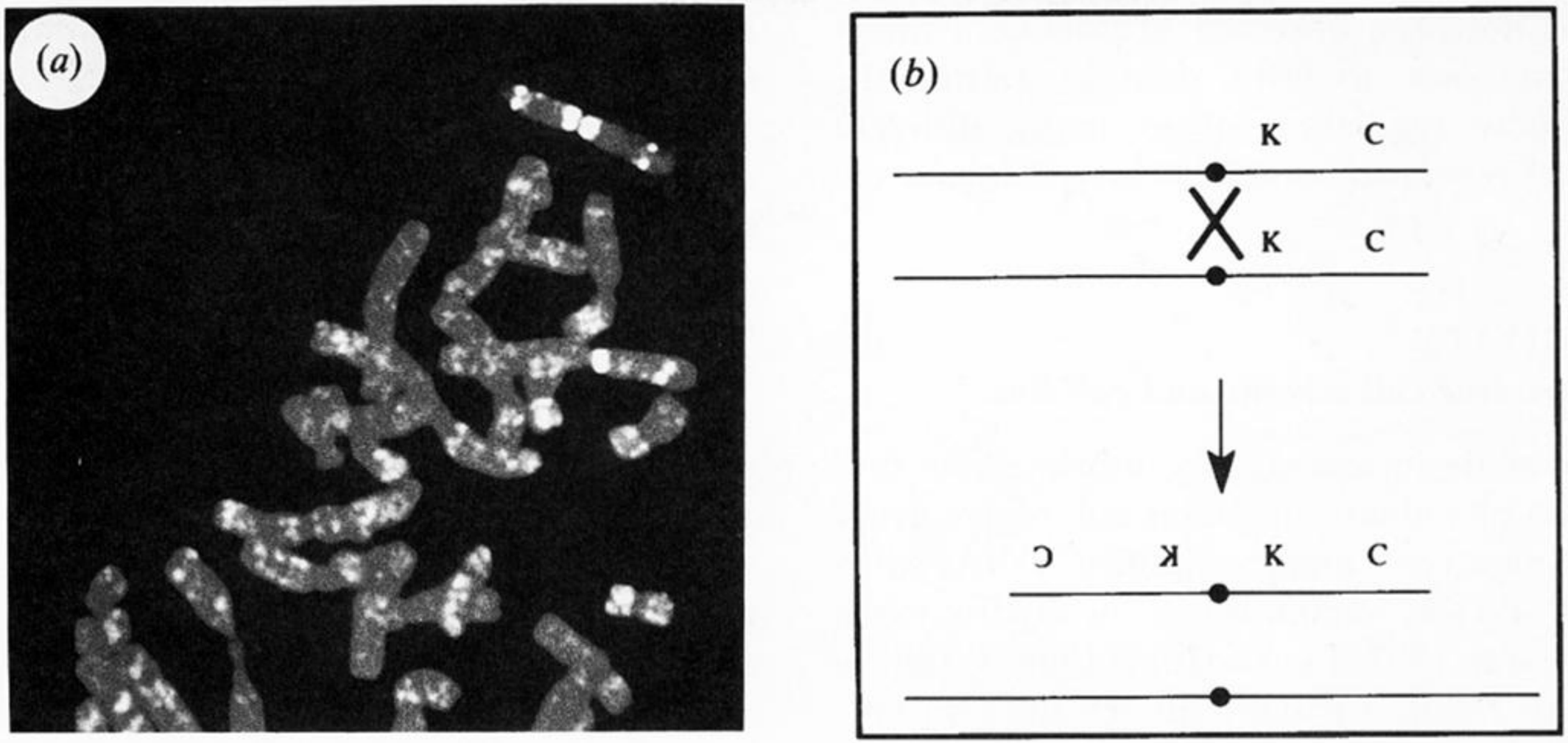

Figure 3. An isochromosome 2p in PALA-resistant HT1080 cells. (*a*) The uppermost chromosome has been derived from human chromosome 2 by centromeric fusion. A normal chromosome 2 can be seen below. The marker chromosome has two p arms with CAD (C) near the telomeres and the V_k gene cluster (K) near the centromere. (*b*) The proposed primary event, involving recombination through the centromeres of sister chromatids. The two centromeres are identical. One has two 'right' portions and the other two 'left' portions. It is important that the recombined centromere on the chromosome bearing CAD retains functions. (Figure and legend reproduced, with permission, from Stark (1993).)