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# Genetic instability in colorectal cancers

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**It has long been considered that genetic instability is an integral component of human neoplasia[1–3]. In a small fraction of tumours, mismatch repair deficiency leads to a microsatellite instability at the nucleotide sequence level[4,5]. In other tumours, an abnormal chromosome number (aneuploidy) has suggested an instability, but the nature and magnitude of the postulated instability is a matter of conjecture. We show here that colorectal tumours without microsatellite instability exhibit a striking defect in chromosome segregation, resulting in gains or losses in excess of $10^{-2}$ per chromosome per generation. This form of chromosomal instability reflected a continuing cellular defect that persisted throughout the lifetime of the tumour cell and was not simply related to chromosome number. While microsatellite instability is a recessive trait[6,7], chromosomal instability appeared to be dominant. These data indicate that persistent genetic instability may be critical for the development of all colorectal cancers, and that such instability can arise through two distinct pathways.**

Aneuploidy occurs frequently in colorectal and other cancers[3]. There are many potential causes for this aneuploidy, and it is unknown whether aneuploidy is associated with a persistent defect in chromosome segregation. We therefore studied eight commonly used colorectal cancer cell lines, four of which were aneuploid and four of which were near diploid. To measure the rate of change in chromosome number, fluorescence *in situ* hybridization (FISH) of interphase cells was performed with a panel of centromeric probes[8]. Clones of each of the eight lines were generated and expanded through a defined number of generations before examination by FISH.

The eight lines could be clearly segregated into two groups based on these analyses (Table 1). Each of the aneuploid lines (HT29, SW480, SW837 and LoVo) showed a dramatic variation in chromosome content among the cells of individual clones. For example, over half of the cells of HT29 clone A gave a variant number of signals of chromosome 7 (either a loss or gain compared to the modal number of 3; Figs 1a and 2). A similar variation affected each of the nine chromosomes examined (Fig. 2 and Table 1). Both losses and gains of chromosomes were observed, with, for example, 34% of cells demonstrating losses and 12% of cells demonstrating gains of chromosome 15 in HT29 clone A (Fig. 2). Evaluation of independent clones of HT29 cells confirmed the reproducibility of these observations (Table 1).

That chromosomal instability (CIN) was not the simple end-product of tumorigenesis was evident from the results obtained with the near-diploid tumour lines, each of which was known to have a different form of instability (MIN, resulting from mismatch repair (MMR) deficiency). For example, most of the cells in HCT116 clone A contained two copies of both chromosomes 7 and 18 (Fig. 1b) and the number of variant HCT116 cells was similar to the background number observed in normal lymphocytes (Table 1). Comparable results were found for chromosomes 1, 3, 4, 8, 12 and 15 in both HCT116 clone A and in independent HCT116 clones, as well as in clones of the other near-diploid lines examined (DLD1, SW48 and RKO; Fig. 2 and Table 1).

As the cells constituting each of the clones evaluated had gone through a similar number of generations and were grown under

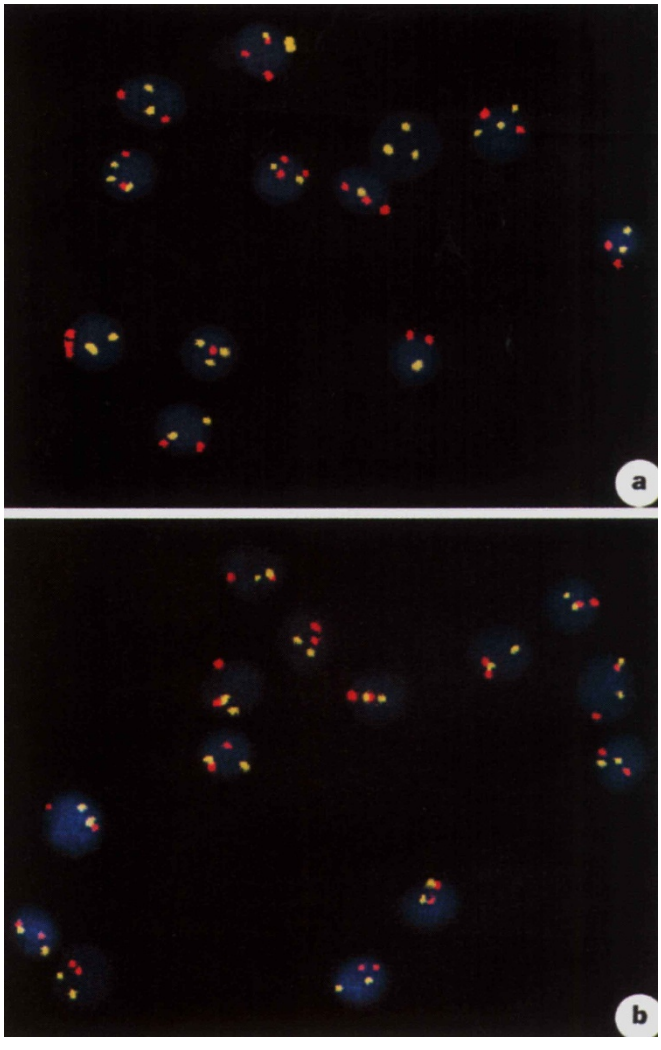


**Figure 1** FISH analysis of colorectal cancer cell lines. Clones of HT29 (**a**) and HCT116 (**b**) were expanded through 25 generations before FISH analysis. Interphase nuclei were hybridized with labelled centromeric DNA probes specific for chromosome 7 (red) and 18 (yellow). HCT116 cells contained two copies of both chromosomes, whereas the number of signals detected in HT29 cells was diverse, indicating chromosomal instability.

**Table 1 Chromosome instability in colorectal cancer cell lines**

| Cell line | | Chrom 1 | Chrom 3 | Chrom 4 | Chrom 7 | Chrom 8 | Chrom 12 | Chrom 15 | Chrom 16 | Chrom 17 | Chrom 18 | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HT29 | M | 3 | 3 | 3 | 3 | 3 | 2 | 3 | ND | 3 | 3 | 2.9 |
| Clone A | F | 54% | 53% | 53% | 55% | 37% | 56% | 46% | ND | 46% | 59% | 51% |
| HT29 | M | 3 | 3 | 3 | 3 | 3 | 2 | 3 | ND | 3 | 3 | 2.9 |
| Clone A/50 | F | 56% | 50% | 48% | 49% | 48% | 53% | 60% | ND | 45% | 49% | 51% |
| HT29 | M | 3 | 3 | ND | 3 | 2 | 3 | 3 | 2 | 3 | 3 | 2.8 |
| Clone B | F | 57% | 48% | ND | 53% | 43% | 30% | 50% | 54% | 33% | 49% | 46% |
| LOVO | M | ND | 4 | 4 | 6 | 4 | 4 | 2 | ND | 3 | 4 | 3.9 |
| Clona A | F | ND | 36% | 25% | 36% | 26% | 44% | 25% | ND | 9% | 30% | 29% |
| SW480 | M | ND | 5 | 4 | 7 | 6 | 6 | 7 | 4 | 5 | 4 | 5.3 |
| Clone A | F | ND | 59% | 30% | 57% | 27% | 41% | 66% | 34% | 37% | 34% | 43% |
| SW837 | M | 2 | 2 | ND | 2 | 3 | 2 | 2 | 2 | ND | 1 | 2 |
| Clone A | F | 48% | 20% | ND | 19% | 24% | 16% | 30% | 33% | ND | 16% | 26% |
| SW837 | M | 2 | 2 | ND | ND | 3 | 2 | 2 | ND | ND | ND | 2.2 |
| Clone B | F | 17% | 32% | ND | ND | 35% | 22% | 28% | ND | ND | ND | 27% |
| HCT116 | M | 2 | 2 | 2 | 2 | 2 | 2 | 2 | ND | ND | 2 | 2 |
| Clone A | F | 6% | 5% | 3% | 3% | 1% | 2% | 4% | ND | ND | 4% | 4% |
| HCT116 | M | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | ND | 2 | 2 |
| Clone A/50 | F | 1% | 7% | 3% | 4% | 1% | 1% | 0% | 3% | ND | 9% | 3% |
| HCT116 | M | 2 | 2 | ND | 2 | 2 | 2 | 2 | ND | 2 | 2 | 2 |
| Clone B | F | 3% | 6% | ND | 1% | 4% | 2% | 5% | ND | 1% | 1% | 3% |
| DLD1 | M | 2 | 2 | ND | 2 | ND | 2 | 2 | ND | 2 | 2 | 2 |
| Clone A | F | 2% | 5% | ND | 12% | ND | 10% | 2% | ND | 2% | 7% | 6% |
| DLD1 | M | ND | ND | ND | 2 | ND | 2 | ND | ND | ND | 2 | 2 |
| Clone B | F | ND | ND | ND | 5% | ND | 7% | ND | ND | ND | 7% | 6% |
| SW48 | M | ND | 2 | ND | 3 | ND | 2 | ND | ND | 2 | 2 | 2.2 |
| Clone A | F | ND | 8% | ND | 2% | ND | 0% | ND | ND | 0% | 6% | 3% |
| RKO | M | 2 | 2 | ND | 2 | ND | 2 | ND | ND | 2 | 2 | 2 |
| Clone A | F | 10% | 9% | ND | 7% | ND | 8% | ND | ND | 10% | 10% | 8% |
| Normal | M | 2 | 2 | ND | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Lymphocytes | F | 1% | 1% | ND | 3% | 4% | 2% | 7% | 2% | 5% | 2% | 3% |

Clones of several cell lines and fusions were expanded through 25 generations before interphase FISH analysis using chromosome-specific centromeric probes. In two cases, HT29 Clone A/50 and HCT116 Clone A/50, the clones were expanded through 50 generations before FISH analysis. For each chromosome tested, the modal chromosome number (*M*) was determined, and the fraction of cells whose chromosome number is different from the mode (*F*), is shown. At least two clones were evaluated for each line studied, with almost identical results in each case; representative results are shown. At least 100 nuclei were evaluated per clone with each chromosome probe. ND, not determined.

identical conditions *in vitro*, the rate of chromosome number change could be directly compared. The non-CIN lines passaged for 50 generations demonstrated far less variation than CIN cells passaged for 25 generations, ruling out trivial differences in generation number as the basis for the noted differences (Table 1). The rate of chromosome loss or gain was estimated to be in excess of $10^{-2}$ per generation for each chromosome analysed in each of the four CIN lines. Corrected for the number of chromosomes per cell and assuming that CIN affects all chromosomes equally, this translated into a gain or loss of a chromosome in at least one of every five cell divisions.

To confirm these estimates of chromosome instability, we developed a method to analyse chromosome variation *in situ* on single colonies containing only ~100 cells. Cell lines were plated at low density on microscope slides so that independent clones could be evaluated after 6–7 generations. FISH was performed following fixation of the colonies without disaggregating them. The cells within such HCT116 colonies showed little chromosome variation (0, 0 and 0.4% variant cells with respect to chromosomes 7, 12 and 17, respectively). HT29 colonies exhibited much greater variation, with 30, 34 and 18% of cells variant for chromosomes 7, 12 and 17, respectively. The degree of chromosome instability calculated from these data is consistent with the estimates generated from later stages of clonal growth (Table 1).

As already noted, all of the lines demonstrating CIN were aneuploid, containing a modal number of 40 (SW837), 87 (LoVo), 119 (SW480) and 71 (HT29) chromosomes, whereas the other four lines were near diploid, each containing 45–47 chromosomes. This observation is compatible with the idea that an underlying CIN caused the observed aneuploidy[2,9]. However, it was equally compatible with the hypothesis that a cell that becomes aneuploid for any reason may thereafter be chromosomally unstable (that is, aneuploidy could cause CIN)[10,11]. To test this possibility, two experiments were done. First, chromosomal stability was tested in a derivative of HCT116 cells in which a single extra copy of chromosome 3 was introduced[6]. As shown in Fig. 2 and Table 2, this derivative was as stable as its parent with respect to all five chromosomes tested, including the extra chromosome 3. Second, chromosomally stable diploid lines were converted into grossly aneuploid ones by fusing drug-marked diploid clones to one another. FISH analysis demonstrated that each fusion line contained the expected number of copies of each chromosome (the sum of the parental numbers). FISH also revealed a distinct difference in chromosome stability between the artificially created aneuploid cells and the naturally occurring aneuploid lines described above. For example, clones derived from HCT116–HCT116 or DLD1–DLD1 fusions each contained a model number of four chromosomes 7, 12 and 18. The variation in the number of these chromosomes among the cells was minimal, like their diploid parents (Fig. 2 and Table 2). As a technical control for these experiments, HT29–HT29 fusions were tested and found to demonstrate the expected high level of chromosomal instability (Fig. 2 and Table 2). Thus an abnormal number of chromosomes in a cell, in and of itself, could not account for the high level of chromosome instability found in the aneuploid colorectal cancers.

Cell fusion experiments also provided a unique opportunity to test whether CIN acted in a dominant or recessive fashion at the cellular level. Most fusions between CIN and non-CIN cells were non-viable, probably because of the existence of complementing tumour-suppressor genes (refs 7, 12, 13, and unpublished data). We

**Table 2 Chromosome instability in colorectal cancer-cell derivatives resulting from fusion**

| Cell line | | Chrom 1 | Chrom 3 | Chrom 7 | Chrom 12 | Chrom 15 | Chrom 17 | Chrom 18 | Average |
|---|---|---|---|---|---|---|---|---|---|
| HCT116 + Ch3 | M | ND | 3 | 2 | 2 | ND | 2 | 2 | 2.2 |
| Clone A | F | ND | 6% | 7% | 3% | ND | 2% | 6% | 5% |
| HCT116 × HCT116 | M | ND | 4 | 4 | 4 | ND | 4 | 4 | 4 |
| Clone A | F | ND | 0% | 2% | 0% | ND | 0% | 1% | 1% |
| HCT116 × HCT116 | M | ND | 4 | 4 | 4 | ND | ND | 4 | 4 |
| Clone B | F | ND | 6% | 4% | 0% | ND | ND | 4% | 4% |
| HCT116 × HCT116 | M | ND | 4 | 4 | 4 | ND | ND | 4 | 4 |
| Clone C | F | ND | 9% | 6% | 4% | ND | ND | 2% | 5% |
| HCT116 × HCT116 | M | ND | 4 | 4 | 4 | ND | ND | ND | 4 |
| Clone D | F | ND | 0% | 0% | 0% | ND | ND | ND | 0% |
| DLD1 × DLD1 | M | ND | ND | 4 | 4 | ND | ND | 4 | 4 |
| Clone A | F | ND | ND | 9% | 8% | ND | ND | 12% | 9% |
| DLD1 × HCT116 | M | ND | ND | 4 | 4 | ND | ND | 4 | 4 |
| Clone A | F | ND | ND | 6% | 7% | ND | ND | 10% | 8% |
| HT29 × HT29 | M | ND | 6 | 6 | 5 | ND | 5 | 6 | 5.6 |
| Clone A | F | ND | 58% | 58% | 58% | ND | 62% | 56% | 58% |
| HT29 × HT29 | M | ND | 6 | 6 | 6 | ND | 5 | 6 | 5.6 |
| Clone B | F | ND | 54% | 52% | 58% | ND | 64% | 56% | 57% |
| HT29 × HT29 | M | ND | 5 | 6 | 6 | ND | 5 | 6 | 5.6 |
| Clone C | F | ND | 70% | 60% | 52% | ND | 60% | 62% | 61% |
| DLD1 × HT29 | M | 5 | ND | 5 | 4 | 5 | 5 | ND | 4.8 |
| Clone A | F | 48% | ND | 58% | 50% | 56% | 48% | ND | 52% |
| DLD1 × HT29 | M | 4 | ND | 5 | 4 | 4 | 5 | ND | 4.4 |
| Clone B | F | 54% | ND | 54% | 42% | 47% | 52% | ND | 50% |
| DLD1 × HT29 | M | 5 | ND | 5 | 5 | 5 | 5 | ND | 5 |
| Clone C | F | 14% | ND | 46% | 44% | 44% | 36% | ND | 28% |
| DLD1 × HT29 | M | 4 | ND | 5 | 5 | 5 | 4 | ND | 4.6 |
| Clone D | F | 55% | ND | 29% | 49% | 64% | 52% | ND | 50% |
| DLD1 × HT29 | M | 5 | ND | 5 | 4 | 5 | 4 | ND | 4.6 |
| Clone E | F | 42% | ND | 34% | 65% | 55% | 54% | ND | 50% |
| DLD1 × HT29 | M | 5 | ND | 5 | 5 | 5 | 5 | ND | 5 |
| Clone F | F | 60% | ND | 33% | 38% | 57% | 44% | ND | 46% |
| DLD1 × HT29 | M | 4 | ND | 5 | 5 | 5 | 5 | ND | 4.8 |
| Clone G | F | 28% | ND | 46% | 53% | 53% | 56% | ND | 47% |
| DLD1 × HT29 | M | 4 | ND | 5 | 5 | 5 | 4 | ND | 4.6 |
| Clone H | F | 32% | ND | 34% | 34% | 50% | 54% | ND | 41% |

Drug-resistant clones were fused with one another as indicated. For example, HCT116 × HCT116 represents the fusion between two clones of HCT116 cells, one marked with geneticin resistance and the other with hygromycin resistance. The line marked HCT116 + Ch3 resulted from transfer of a normal human chromosome 3 to HCT116 cells[6]. This derivative was tested in the absence of selection for the introduced chromosome 3 (which contained a geneticin-resistance marker gene). Modal chromosome numbers (*M*) and variant fractions (*F*) were determined as for Table 1. At least two clones were evaluated for each fusion and the stability levels were found to be very similar among independent clones; representative results are shown. At least 100 nuclei were evaluated per clone. ND, not determined.

noticed, however, that both HT29 cells (a CIN line) and DLD1 cells (a MIN line) had similar tumour-suppressor gene alterations, including inactivating mutations of the APC and p53 genes[14–16]. We therefore predicted that fusions between HT29 and DLD1 lines would be viable, and this turned out to be the case. The MIN phenotype of DLD1 was corrected by the introduction of the HT29 cell genome, as expected for a 'recessive' trait. In contrast, the CIN phenotype of HT29 persisted in the hybrid cells. In each of 9 such clones tested, chromosomal variation was high, with variant frequencies averaging 48% for five different chromosomes, similar to the HT29 parental cells (Table 2). Controls for these experiments were provided by fusions of non-CIN cells, including DLD1 cells, to one another; each of 13 independent clones were evaluated, and each remained chromosomally stable, despite the induced aneuploidy (examples are shown in Table 2). These results indicate that the CIN phenotype of HT29 cells acts dominantly at the cellular level, at least in the DLD1 background.

Our results strongly support the view that aneuploidy in cancers reflects an underlying chromosomal instability[2,9]. Though aneuploidy is sometimes equated with instability, it is important to note that aneuploidy is a state, not a rate, and aneuploidy could in theory result from many factors other than a persistently elevated rate of chromosomal change. For example, an abnormal or heterogeneous chromosome number in a cancer cell could result from a small number of catastrophic mitoses during tumour evolution in the absence of any persistent underlying defect in chromosomal segregation. Similarly, aneuploidy could result from the preferential outgrowth of cells with an abnormal chromosome number, selecting for gains of chromosomes containing activated oncogenes and for losses of chromosomes containing tumour-suppressor genes. Such selection could lead to aneuploidy in the absence of any change in the underlying *rate* of chromosomal gain or loss. Our results exclude these possibilities through the demonstration of genuine differences in the rates of chromosomal gain and loss in cancers with CIN, compared to that in normal cells or in cancer cells with MIN. They also demonstrate that, unlike the situation in yeast[10], induced aneuploidy in human cells does not intrinsically lead to a chromosome instability.

It thus appears that all colorectal cancers may be genetically unstable, but that this instability can arise through one of two different pathways. Our results are consistent with karyotypic observations demonstrating that mismatch repair-deficient tumours, accounting for ~15% of colorectal cancers (CRC) are often diploid, whereas other CRC are generally aneuploid[17,18]. Only one of the eight lines studied (LoVo) was both MIN and CIN, suggesting that either type of instability may be sufficient for driving

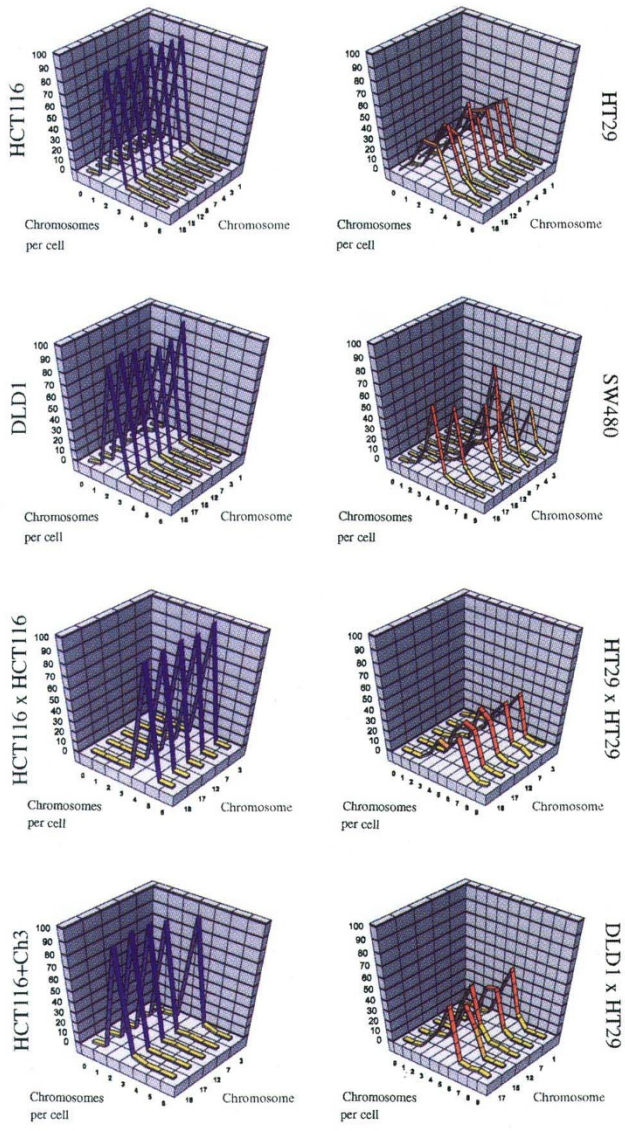


**Figure 2** Ploidy and chromosomal instability. Clones of several cell lines and fusions were expanded through 25 generations, then analysed by FISH using chromosome specific centromeric probes. For each chromosome indicated, the fraction of cells with the indicated number of signals per nucleus is plotted along the *z*-axis. Blue ribbons represent fractions >90%; shades of red indicate fractions less than 75%. At least 100 nuclei were evaluated per clone for each chromosome tested.

the neoplastic process, but that MIN does not preclude CIN and vice versa. Although *p53* gene mutations have been invoked as a cause of some forms of genetic instability[19,20], such mutations are unlikely to be responsible for CIN, as there was no simple relationship between p53 mutation and the CIN phenotype. For example, p53 is mutant in the chromosomally stable DLD1 cells[15], but is wild-type in the chromosomally unstable LoVo line[21]. We suggest that a gene affecting chromosome segregation is mutated in a dominant fashion early during colorectal tumorigenesis, and that the resultant instability drives the tumorigenic process in much the same way as MIN drives neoplasia in mismatch repair-deficient tumours. Further understanding of the genetic and biochemical mechanisms underlying this very common form of instability should prove important for understanding the tumour heterogeneity that is so central to cancer biology and treatment. □

## Methods

**Cell culture.** Cells were cultured in McCoy's 5A medium supplemented with 10% fetal bovine serum (Hyclone Lab), 100 U ml$^{-1}$ penicillin, and 0.1 mg ml$^{-1}$ streptomycin. Monolayer cultures were grown at 37 °C in a 5% $CO_2$ atmosphere. The cell lines DLD1, HCT116, HT29, LoVo, SW48, SW480 and SW837 were obtained from ATCC (Rockville, MD), and RKO was generously provided by M. Brattain. Their status with respect to microsatellite instability has been analysed previously[15,22–24]. The cell lines DLD1, HT29, SW480 and SW837 contained mutant *p53* genes, whereas HCT116, LoVo, SW48 and RKO were *p53* wild-type (refs 14, 15, 21, 25, and our unpublished data). Cells were transfected with pCEP4 or pCEP9 using lipofectamine (Gibco BRL) and selected for resistance to geneticin or hygromycin, respectively. The resultant clones were evaluated for chromosomal instability between 25 and 50 generations following transfection.

**Fluorescence *in situ* hybridization.** Centromeric probes specific for chromosomes 1, 3, 4, 7, 8, 12, 15, 16, 17 and 18 were labelled with biotin–16-dUTP or digoxigenin–11-dUTP by nick translation[8]. Nuclei were fixed on slides and pretreated with RNase and pepsin as described[26]. In some experiments (see text), cells were grown directly on microscopic slides and fixed in 3:1 methanol/acetic acid (vol/vol). Multicolour FISH was performed by following standard procedures[27]. In most of the experiments, two differently labelled probes were hybridized and detected simultaneously. Biotinylated probes were detected with FITC avidin–DCS. Digoxigenin-labelled probes were detected with TRITC-conjugated rabbit–anti-mouse and goat–anti-rabbit antibodies. Cells were counterstained with DAPI. Evaluation of interphase nuclei was performed by standard epifluorescence microscopy (Zeiss Axiophot)[28]. Photographs were taken with the 64 × objective using a CCD camera (Photometrics) equipped with a Kodak KAF 1400 chip. For digital image acquisition in the TIFF format, software package 'Nu200 2.0' (Photometrics) was applied. Further processing of images, which included only pseudocolouring and merging, was performed with the software package Gene Join[29].

**Cell fusion.** About $5 \times 10^6$ cells from transfected, drug-resistant clones (geneticin or hygromycin) were combined and resuspended in 1 ml 50% PEG-1450 in McCoy's 5A medium and incubated at 37 °C for 1 min, then washed in 20 ml McCoy's 5A. Cells were incubated overnight in growth medium, and drug selection (double selection with geneticin and hygromycin) started the following day. Doubly resistant fusion clones were isolated after 3 weeks.

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# Activation of prokaryotic transcription through arbitrary protein–protein contacts

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**Many transcriptional activators in prokaryotes are known to bind near a promoter and contact RNA polymerase[1–5], but it is not clear whether a protein–protein contact between an activator and RNA polymerase is enough to activate gene transcription. Here we show that contact between a DNA-bound protein and a heterologous protein domain fused to RNA polymerase can elicit transcriptional activation; moreover, the strength of this engineered protein–protein interaction determines the amount of gene activation. Our results indicate that an arbitrary interaction between a DNA-bound protein and RNA polymerase can activate transcription. We also find that when the DNA-bound 'activator' makes contact with two different components of the polymerase, the effect of these two interactions on transcription is synergistic.**

We replaced the carboxy-terminal domain (CTD) of the α-subunit of RNA polymerase (RNAP) (Fig. 1), which is the natural target for many transcriptional activators[1–3], with a heterologous protein domain that does not ordinarily mediate transcriptional activation. To do this, we took advantage of the well defined properties of the CTD of the bacteriophage λ cI protein (λcI).

λcI is a two-domain protein that binds DNA as a dimer, and pairs of dimers bind cooperatively to adjacent operator sites (Fig. 2a)[5]. The amino-terminal domain (λcI-NTD) contacts the DNA and can interact with the σ subunit of RNAP, stimulating transcription when λcI is bound at promoter $\lambda P_{RM}$ (refs 6, 7). The λcI-CTD mediates both dimer formation and the dimer–dimer interaction that results in cooperativity (reviewed in ref. 8) (Fig. 2a). λcI mutants specifically defective for transcriptional activation bear amino-acid substitutions in the λcI-NTD[9], and λcI mutants specifically defective for cooperative binding to DNA bear amino-acid substitutions in the λcI-CTD (ref. 10 and refs therein).

We reasoned that if we replaced the α-CTD with the λcI-CTD, the resulting α-cI chimaera would display a dimeric target that could be contacted by an appropriately positioned λcI dimer (Fig. 2b). We wanted to investigate whether the same protein–protein interaction that ordinarily mediates the cooperative binding of pairs of λcI dimers to the DNA would mediate transcriptional activation when the λcI-CTD is tethered to the α-NTD.

We constructed a derivative of the *lac* promoter termed *plac* $O_R2$-62, bearing a single λ operator ($O_R2$) centred 62 base pairs (bp) upstream of the transcription startpoint (at −62) (Fig. 2b) and introduced it in single copy into the chromosome of *Escherichia coli* strain MC1000 F′ *lacI*$^q$ to create strain KS1. As expected, λcI alone does not activate transcription from *plac* $O_R2$-62 (Fig. 3a), because when bound this far away from the promoter it cannot contact the σ-subunit of RNAP. However, λcI stimulated transcription in the presence of the α-cI chimaera approximately 10-fold, as measured by β-galactosidase assay (Fig. 3a). This stimulation was not dependent on the natural activating region located in the λcI-NTD (data not shown). In the absence of the α-cI chimaera, λcI repressed transcription slightly. Furthermore, in the absence of λcI, expression of the α-cI chimaera had no significant effect on transcription from *plac* $O_R2$-62. Primer extension analysis confirmed that λcI stimulated the production of correctly initiated transcripts (Fig. 3c).

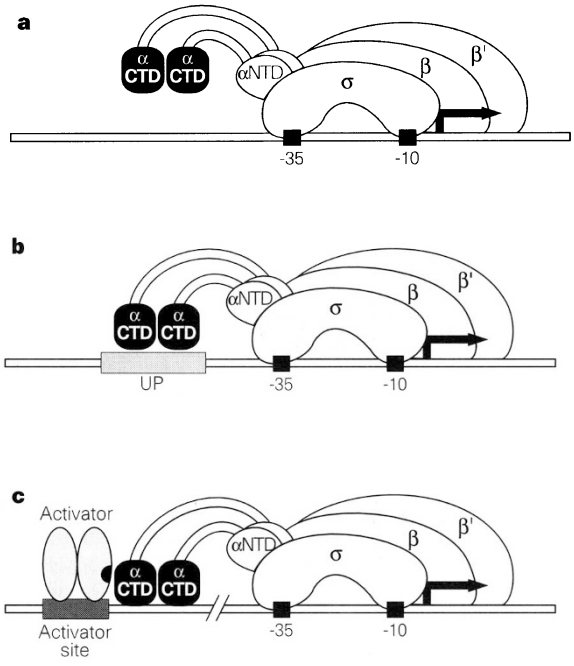


**Figure 1** Function of the α-CTD in transcriptional activation. **a**, Basal transcription from a promoter that does not have an associated upstream element or transcriptional activator binding site. RNAP in *E. coli* consists of an enzymatic core composed of subunits α, β and β′ in the stoichiometry $\alpha_2\beta\beta'$, and one of several alternative σ factors responsible for specific promoter recognition. **b**, Stimulated transcription from a promoter bearing an associated upstream element (UP) involves specific protein–DNA interaction between the α-CTD and the UP element. The α-CTD, which is tethered to α-NTD by a flexible linker region, can interact with the DNA UP element that is found upstream of the −35 region of certain particularly strong promoters[30]. **c**, Stimulated transcription from a promoter bearing an associated activator binding site involves specific protein–protein interaction between the α-CTD and the DNA-bound activator.

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