Correlation between interphase and metaphase chromosome arrangements as studied by laser-uv-microbeam experiments*

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In spite of many attempts at resolution, the problem of the chromo-
some topography in interphase nuclei and its possible functional
significance (Lewin, 1981; Vogel and Krüger, 1983) has remained a
matter of great controversy and uncertainty (for reviews see Co-
mings,1980; Avivi & Feldman, 1980). In particular, the relationship
between metaphase and interphase arrangements of chromosomes is
still largely unknown. A solution of this problem seems especially
urgent, since the spatial relationship of chromosomes at metaphase
has been extensively studied in many species during the last twenty
years. Recently, Bennett and coworkers have demonstrated a highly
ordered arrangement of centromeres in metaphase plates of certain
plant species (Bennett, 1983) and stimulated fresh interest in the
development of techniques for mapping the arrangements of indivi-
dual chromosomes directly within the interphase nucleus. In this
paper we describe a laser-uv-microbeam approach which allows selec-
tive microirradiation of a small amount of chromatin in the inter-
phase nucleus of cultured cells and study of its distribution in
chromosomes during the subsequent mitosis. Alternatively, chromatin
can be microirradiated during mitosis and its distribution can be
studied in the subsequent interphase nucleus.

TERRITORIAL ORGANIZATION OF CHROMOSOMES IN THE INTERPHASE NUCLEUS
OF CULTURED CHINESE HAMSTER CELLS

A prerequisite to any meaningful conclusion which might be
drawn from studies of chromosome arrangements at metaphase, on the
arrangement of chromosomes in the interphase nucleus, is the as-
sumption that each chromosome continues to exist in the interphase
nucleus as a spatially distinct, and in spite of its decondensa-
tion, still relatively compact entity. In this case, each single
chromosome would occupy a small and coherent domain of the total
nuclear space. The hypothesis that such chromosome territories
(also called chromosome domains) exist in the interphase nucleus
has been put forward already by Rabl (1885) and Boveri (1909).
Other models, however, proposed half a century later, have still
seriously considered the possibility that the chromatin fibres of
euchromatic regions within individual chromosomes might become
extensively dispersed in the interphase nucleus (Comings, 1968;
Vogel and Schroeder, 1974). In case that the chromatin fibre, which
is supposed to constitute each individual chromosome, would be
extended throughout a major part of the total nuclear space, each
chromosome would form associations with many other chromosomes at
many sites. If so, chromosome arrangements at metaphase would
naturally yield an insufficient picture of their interphase arran-
gements.

Laser-uv-microirradiation ($\lambda = 257$ nm) has provided the possi-
bility to put such models to a rigorous test. Small parts of inter-
phase nuclei of cultured Chinese hamster cells were microirradia-
ted. Obviously, the distribution of microirradiated chromatin in
the chromosomes of the subsequent metaphase critically depends on
the actual arrangement of interphase chromosomes in the microirra-
diated nuclear part. In the case of a territorial organization, it
is predicted that microirradiated chromatin is restricted to a few
metaphase chromosomes, depending on the size of the microirradiated
nuclear segment. In contrast, extensive dispersion of the chromatin
fibres from individual chromosomes should be indicated by scatte-
ring of microirradiated chromatin across a correspondingly increa-
sing number of metaphase chromosomes.

Microirradiated chromatin was identified either autoradiogra-

phically by detection of unscheduled DNA synthesis (UDS) which takes place during the course of excision repair of DNA photolesions in the microirradiated nuclear segment (Zorn et al., 1979; Cremer et al., 1982a) or by use of antibodies specific for the detection mainly of pyrimidine dimers in a DNA structure which
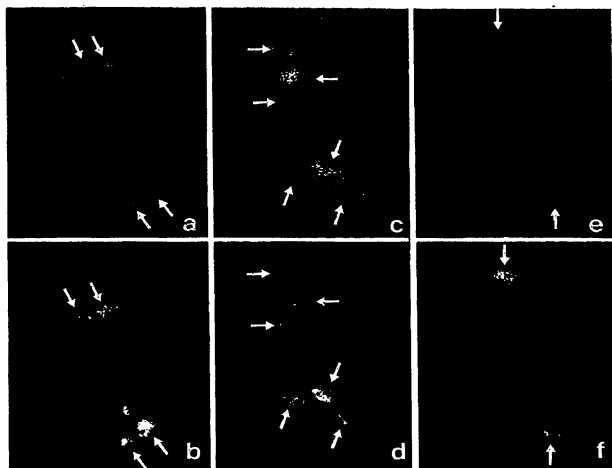

Fig. 1: Pairs of daughter nuclei were fixed in acetic acid/methanol (1:3) 3h 45' a, 3h 25' c and 4h 25' e after microirradiation of a small part of the metaphase plate of Chinese hamster cells (M3-1). a,c,e Nuclei stained with DAPI. b,d,f Immunocytochemical localization of microirradiated chromatin (arrows). Staining for indirect immunofluorescence microscopy was performed as previously described (Cremer et al., 1983). Antibodies with a high affinity for uv-irradiated DNA were raised independantly in rabbits by Dr. K. Nakanishi and Dr. J.J. Cornelis. Both antisera yielded identical results. Goat-anti-rabbit IgG conjugated with fluoresceine isothiocyanate (FITC) was obtained from Nordic. For comparison nuclei were counterstained with DAPI, thereafter. By appropriate filtering, the blue DAPI fluorescence could easily be distinguished from the green immunofluorescence. Microphotopraphs were taken with a Zeiss photomicroscope equipped with epifluorescence illumination. A mirror-like distribution of microirradiated chromatin in each pair of daughter nuclei becomes more obvious when changes in the positions of the whole nuclei during the postincubation period are taken into consideration and nuclei are accordingly repositioned.

represent the major DNA-photoproduct of uv-irradiation at 257 nm (Cremer et al., 1983; Hens et al., 1983). With both methods of detection we found that microirraditation of a small part of the interphase nucleus (approx. 5 % of the total nuclear area) in different lines of Chinese hamster cells, resulted in intensive labelling of segments from a few chromosomes only (3 to 4 on the average), while the majority of chromosomes in each metaphase spread (modal numbers 22 - 23) remained unlabelled. It is important to note that this result was obtained both after microirradiation of nuclei in G1 (Cremer et al., 1982a) and in S-phase (Hens et al., 1983), indicating that a territorial organization of chromosomes is maintained during subsequent stages of the cell cycle. Furthermore, it was found that the number of chromosomes in which formation of sister chromatid exchanges (SCEs) was induced at a given uv-incident energy increased significantly with the size of the microirradiated nuclear area (Raith et al., 1984). In addition, mitotic chromosomes were microirradiated either at metaphase (see below) or at anaphase (Hens et al., 1983). In the subsequently formed interphase nuclei the microirradiated chromatin was limited to a small volume (Fig. 1). In conclusion these microbeam experiments support the concept that interphase chromosomes occupy distinct domains of the interphase nucleus.

MIRROR-LIKE DISTRIBUTION OF CHROMATIN IN PAIRS OF DAUGTHER NUCLEI

Microirraditaion of metaphase chromosomes in living Chinese hamster cells allowed us to test a hypothesis which was proposed for the first time by Theodor Boveri (1888; 1909). Boveri stated that arrangements of chromosomes in pairs of daughter nuclei would occur in a mirror-like fashion. This hypothesis was based on observations made during the first cell cycles of fertilized eggs of Ascaris megalocephala. Boveri found that the arrangements of prophase chromosomes were mirror like in each pair of daughter cells derived from the same mitotic event, while they appeared variable to a large extent in cells from different mitotic events. By means of immunofluorescence microscopy we have visualized the distribution of microirradiated chromatin in a sample of 27 pairs of daugh-

ter nuclei obtained after microirradition of a small part of the
metaphase plate (see Fig. 1 for examples). To test the similarity
of the chromatin distribution in each pair, the daughter nuclei
were projected over each other in a mirror like fashion and positio-
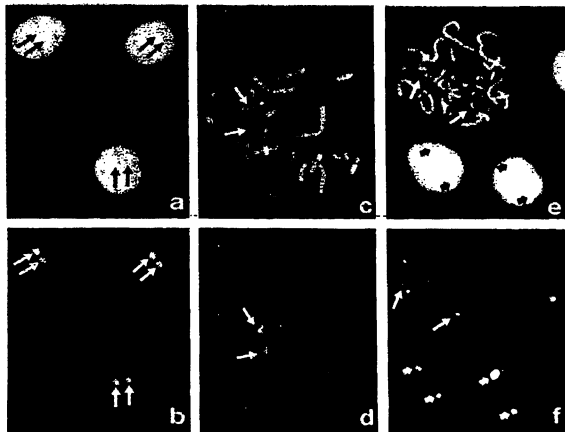

Fig. 2:   Microirradiation of interphase nuclei at two sites of
Chinese hamster cells (M3-1) was performed six hours after release
of the cells from a thymidine block (2 mM). Chromosome preparations
were performed in situ as previously described (Zorn et al., 1979)
after a postincubation period of 4 hours in the presence of caffei-
ne (2 mM). a,c,e Interphase nuclei and metaphase spreads stained
with DAPI. b,d,e Immunocytochemical localization of microirradiated
chromatin. (For details see legend to Fig. 1) Arrows indicate
corresponding sites of microirradiated chromatin in a,b, c,d and
e,f. a,b Microirradiation at two nuclear sites close to each
other. c,d Metaphase spread obtained after microirradiation of the
nucleus in the preceding interphase at two sites close to each
other. e,f Metaphase spread obtained after microirradiation of the
interphase nucleus at two sites far apart from each other as demon-
strated by two adjacent nuclei. Notably, two sites of indirect
immunofluorescence corresponding with two sites of partial chromo-
some shattering (PCS) (Cremer et al., 1983) can be detected in the
two metaphase spreads. In contrast, after microirradiation of the
interphase nucleus at one site indirect immunofluorescence corres-
ponding to one site of PCS was generally observed in the subsequent
metaphase (Hens et al., 1983).

ned in a way that yielded an overlap of both the total nuclear
areas and the immunoflourescent areas as complete as possible. For
comparison, 27 pairs of non-daughter nuclei of similar size were
tested from the same sample. In support of Boveri's hypothesis, we
found that the extent to which the immunofluorescent areas overlap-
ped was significantly larger (by a factor of approx. 2) in pairs of
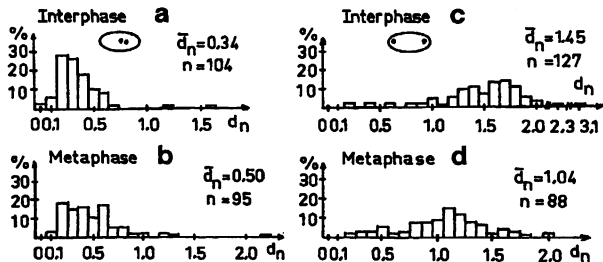daughter nuclei. (K. Nakanishi & T. Cremer, in preparation).

Fig. 3: Comparison of relative distances d_n in interphase a,c and
metaphase b,d between the two sites of microirradiated chromatin in
Chinese hamster cells (M3-1) (for further details compare Fig. 2).
In each interphase nucleus and metaphase spread the distance d
between the centers of the two immunofluorescent sites correspon-
ding to the two sites of microirradiated chromatin was determined.
Nuclei and metaphase spreads were considered as ellipses and the
diameters of the two major axes a and b were also measured. To
avoid any prejudice, evaluation of metaphase spreads was performed
by a person without information on the particular type of microirr-
adiation. To account for variations in size of nuclei and metaphase
spreads d was divided by $1/2\sqrt{ab}$ in each case. $1/2\sqrt{ab}$ corresponds to
the radius of a circle of the same area. a) Frequency distribution
of d_n in a sample of 104 interphase nuclei with two sites of micro-
irradiation close to each other. Nuclei were situated close to the
metaphase spreads evaluated in b (compare Fig. 2 a,b). b) Frequency
distribution of d_n in 95 metaphase spreads obtained 4-6 hours after
microirradiation of nuclei in 4419 cells at two sites as described
in a (compare Fig. 2 c,d). c) Frequency distribution of d_n in a
sample of 127 interphase nuclei situated close to metaphase spreads
evaluated in d and microirradiated at two sites far apart from each
other. d) Frequency distribution of d_n in 88 metaphase spreads
obtained 4-6 hours after microirradiation of the interphase nucleus
in 4350 cells as described in c (compare Fig. 2 e,f). Note that the
mean values of d_n in metaphase spreads still reflect the distances
between the two sites of microirradiation in the interphase nuc-
leus. The difference between d_n in a,c (interphase nuclei) and b,d
(metaphase spreads) is highly significant (P<0.001)

STATIC ARRANGEMENT OF CHROMOSOME TERRITORIES IN INTERPHASE NUCLEI
OF CULTURED CHINESE HAMSTER AND HUMAN FIBROBLASTOID CELLS

Theodor Boveri (1909) was also the first to predict that the
chromosome territories would be rather rigidly fixed during inter-
phase. Notably, cell cycle transit times in the developing egg of
<u>Ascaris</u> <u>megalocephala</u> from which Boveri's hypothesis was derived
are rather short and many decades later the possibility of extensi-
ve alterations in chromosome order during subsequent stages of the
cell cycle was still taken into consideration as one possibility
among others for other cell types (Vogel and Schroeder, 1974). In
case of a dynamic interphase chromosome arrangement, studies of
metaphase chromosome arrangements could at best provide information
on interphase chromosome arrangements at a late stage of the cell
cycle. We, therefore, decided to reinvestigate this problem both in
fibroblastoid Chinese hamster cells (CHL) (Cremer et al., 1982a,b)
and in human fibroblastoid cells (Flow 2000; population doubling
level approx. 30). Interphase nuclei were microirradiated at two
sites and cells were either fixed immediately thereafter or allowed
to grow for an additional postincubation period (20 and 40 hours in
case of CHL-cells and 10 and 20 hours in case of human cells).
Microirradiated chromatin was made visible as described above
either by UDS-labelling or by immunofluorescent labelling. Our data
are consistent with Boveri's early hypothesis. Distances between
the two sites of microirradiated chromatin changed only slightly in
CHL-cells (Cremer et al., 1982 a and appeared practically constant
in human cells (K. Nakanishi & T. Cremer, in preparation).

CORRELATION BETWEEN CHROMOSOME POSITIONS IN INTERPHASE AND
METAPHASE

The arrangement of chromosome territories in the interphase
nucleus is expected to differ from their order in metaphase prepa-
rations. Factors which disrupt the interphase order include conden-
sation of chromosomes during prophase and chromosome movements
involved in the metaphase plate formation. In addition, techniques
routinely used to obtain metaphase spreads suitable for karyotyping
(including spindle poisons, hypotonic treatment and spreading of

chromosomes) may destroy the chromosome arrangement in the intact metaphase plate to an extent which is difficult to control. It should also be noted that chromosomes in a metaphase spread are arranged in a plane, while their respective territories were arranged in the threedimensional structure of the preceding interphase nucleus. In spite of these limitations a statistical evaluation of distances between metaphase chromosomes has been attemped by many authors, and conflicting results have been published. Therefore, we found it important to investigate whether such distances would reflect, if only to a limited extent, the distances between the respective chromosome territories in the preceding interphase. If such a correlation could indeed be demonstrated even for metaphase spreads obtained by routine preparation procedures, the case of the metaphase plate as a substitute for the investigation of interphase chromosome arrangements could be strenghtened, especially under conditions which do not disrupt its natural arrangement by crude spreading techniques.

Until recently, the possiblity to study such a correlation was restricted to a few cases in which heterochromatic regions of particular chromosomes could be studied in mitosis as well as in interphase (e.g. Pera & Schwarzacher, 1970). As a general test of this correlation we have microirradiated two sites in interphase nuclei of Chinese hamster cells (M3-1); either close to or far apart from each other. Microirradiated chromatin served as a probe to study its respective position in metaphase preparations by indirect immunofluorescence microscopy (Figs. 2 and 3; see legends for further experimental details). Both interphase nuclei and metaphase preparations consistantly revealed two distinct sites of immunofluorescent staining (Fig. 2). Distances between these two sites were measured in enlarged photographic prints and corrected with regard to the varying diameters of nuclei and metaphase spreads (see legend to Fig. 3). The results of these measurements show that the mean distances between the two sites of microirradiated chromatin in the interphase nucleus are reflected by their corresponding distances in the subsequent metaphase spread. Another series of experiments was performed in essentially the same way except that metaphase plates were fixed with acetic acid/methanol

(1:3) without colchicine pretreatment and hypotonic shock. Prelimi-
nary data of this series further support the concept that chromoso-
me order in the undisturbed metaphase plate might indeed reflect
the relative positions of chromosome territories in the preceding
interphase nucleus to a considerable extent. A full account of
these experiments will be published elsewhere (H. Baumann, T.
Cremer, N. Bulbuc & J.J. Cornelis, in preparation).

Fig. 2 c-f clearly shows shattering of metaphase chromosomes
corresponding with the sites of immunofluorescent staining. We have
termed this effect as partial chromosome shattering (PCS) (Cremer
et al., 1983) and suggest that it might at least partly be due to
an undercondensation of microirradiated chromosome segments. This
in turn could explain why several chromosomes microirradiated at
one site of the interphase nucleus were often closely associated at
one site of PCS in the subsequent metaphase (Hens et al., 1983).
Shattering resulted from the synergistic effect of caffeine (2mM)
on chromosome damage in microirradiated cells (Cremer et al.,
1983). When caffeine was omitted from the postincubation medium
chromosomal damage was much less pronounced. However, immunofluo-
rescent labelling in these metaphase spreads generally was too weak
for an unequivocal identification of the microirradiated chroma-
tin. Possibly, antigenic sites become masked when microirradiated
chromosomes condense normally in the absence of caffeine.

MAPPING OF INTERPHASE POSITIONS OF INDIVIDUAL CHROMOSOMES

In a recently published series of experiments (Cremer et al.,
1982 b), the interphase nucleus of CHL-cells was microirradiated at
one site either at its central part or at its perhiphery. Metaphase
spreads with PCS obtained after postincubation with caffeine (0.5
mM) were karyotyped. No significant difference was observed between
the frequencies of damage obtained for individual chromosomes at
either site of microirradiation. Interestingly, while an average of
5 - 6 damaged chromosomes per metaphase was found in these experi-
ments, the frequency of joint damage of homologous chromosomes was
low, arguing against somatic pairing of homologuos chromosomes as a
frequent event in this cell type. In spite of their limitation,

these data demonstrate the feasibility of the microbeam approach for mapping the posititons of individual chromosomes in the interphase nucleus.

Avivi, L. and Feldman, M. 1980. Arrangement of chromosomes in the interphase nucleus of plants. Hum. Genet. 55, 281-295.

Bennett, M.D. 1983. The spatial distribution of chromosomes. In Kew Chromosome Conference II, P.E. Brandham and M.D. Bennett eds., 71-79, London: George Allen & Unwin.

Boveri, Th. 1888. Zellen Studien. Jena. Z. Naturw. 22, 687-882.

Boveri, Th. 1909. Die Blastomerenkerne von Ascaris megalocephala und die Theorie der Chromosomenindividualität. Arch. Zellforsch. 3, 181-268.

Comings, D.E. 1968. The rationale for an ordererd arrangement of chromatin in the interphase nucleus. Am. J. Hum. Genet. 20, 440-460.

Comings, D.E. 1980. Arrangement of chromatin in the interphase nucleus. Hum. Genet. 53, 131-143.

Cremer, T., Cremer, C., Baumann, H., Luedtke, E.-K., Sperling, K., Teuber, V. and Zorn, C. 1982 a. Rabl's model of the interphase chromosome arrangement tested in Chinese hamster cells by premature chromosome condensation and laser-uv-microbeam experiments. Hum. Genet. 60, 46-56.

Cremer, T., Cremer, C., Schneider, T., Baumann, H., Hens, L. and Kirsch-Volders, M. 1982b. Analysis of chromosome positions in the interphase nucleus of Chinese hamster cells by laser-uv-microirradiation experiments. Hum. Genet. 62, 201-209.

Cremer, C., Cremer, T., Hens, L., Baumann, H., Cornelis, J.J. and Nakanishi, K. 1983. UV microirradiation of the Chinese hamster cell nucleus and caffeine post-treatment. Immunocytochemical localization of DNA photolesions in cells with partial and generalized chromosome shattering. Mutat. Res. 107, 465-476.

Hens, L., Baumann, H., Cremer, T., Sutter, A., Cornelis, J.J. and Cremer, C. 1983. Immunocytochemical localization of chromatin regions uv-microirradiated in S-phase or anaphase. Evidence for a territorial organization of chromosomes during cell cycle of cultured Chinese hamster cells. Exp. Cell Res., in press.

Lewin, R. 1981. Do chromosomes cross talk? Science 214, 1334-1335.

Pera, F. and Schwarzacher, H.G. 1970. Localization of the heterochromatic chromosomes of Microtus agrestis in interphase and mitosis. Cytobiology 2, 188-199.

Rabl, C. 1885. Über Zelltheilung. Morph. Jb. 10, 214-330.

Raith, M., Cremer, T., Cremer, C. and Speit, G. 1984. Sister chromatid exchange (SCE) induced by laser-uv-microirradiation. Correlation between the distribution of photolesions and the distribution of SCEs. Submitted for publication.

Vogel, F. and Schroeder, T.M. 1974. The internal order of the interphase nucleus. Hum. Genet. 25, 265-297.

Vogel, F. and Krüger, J. 1983. Is there a general relationship between estimated chromosome distances in interphase and location of genes with related functions? Hum. Genet. 63, 362-368.

Zorn, C., Cremer, C., Cremer, T. and Zimmer, J. 1969. Unscheduled DNA synthesis after partial UV-irradiation of the cell nucleus. Distribution in interphase and metaphase. Exp. Cell Res. 124, 111-119.