EVIDENCE FOR SUBCHROMATID ORGANIZATION IN MARSUPIAL CHROMOSOMES

I. Light and Electron Microscopy of X-Ray–Induced Side-Arm Bridges

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INTRODUCTION

The exposure of mitotic or meiotic cells to ionizing radiation or certain drugs produces chromosome aberrations which appear as side-arm bridges at anaphase. The aberration has been so named because the bridge apparently involves a chromosomal strand smaller than either of the anaphase chromosome arms, i.e., a subchromatid exchange (Nebel, 1936; Swanson, 1947; Crouse, 1954; LaCour and Rutishauser, 1954). Östergren and Wakonig (1954), on the other hand, challenged the contention that side-arm bridges are formed by subchromatic exchanges. These investigators were unable to observe chromatid breaks in the second mitosis following irradiation in *Allium cepa*, as might be expected if half chromatid breaks had occurred before replication. They proposed that the side-arm configurations were pseudochiasmata resulting from "stickiness" of the chromosomal matrix. A similar interpretation was made by Kihlman and Hartley (1967) in *Vicia faba*. Peacock (1961), however, reported that such aberrations induced in chromosomes of *Vicia faba* did express as chromatid breaks in the subsequent division. Peacock's observations are supported by Heddle (manuscript in preparation) who also found chromatid aberrations in *Vicia faba* following an intervening period of DNA replication after irradiation. More recently, Whissel and Heddle (personal communication) observed the induction of side-arm bridges in rat kangaroo cells with X-irradiation and have found that such aberrations appeared as chromatid breaks following a subsequent period of DNA replication.

Since the structure of the side-arm bridge may have significance in interpreting the organization of metazoan chromosomes, the ultrastructure of radiation-induced side-arm bridges was investigated in detail.

MATERIALS AND METHODS

The procedure of Brinkley et al. (1967) which permits combined light and electron microscopy of selected structures of mammalian cells in culture was used for the study of X-ray-induced side-arm bridges in rat kangaroo cells (strain PtK_1). This method was particularly suitable since it did not involve the use of mitotic inhibitors or other drugs which might confuse the interpretation. Monolayer cultures were grown in McCoy's 5a medium supplemented with 20% fetal calf serum. Approximately 1×10^6 cells were seeded in Falcon plastic Petri dishes (30 ml) and incubated overnight at 37°C. The cells were exposed to a dose of 250 rads of X-rays from a G.E. "Maxitron" (General Electric Co.,) unit operating at 250 Kev (peak), 15 ma, filtered with 0.5 mm of Cu and 1.0 mm of Al, giving a half value layer of 1.26 mm of Cu. The dose rate was 180 rads per min.

The cells were returned to the 37°C incubator for 30 min following irradiation and then fixed in 3% phosphate-buffered glutaraldehyde at room temperature for 1 hr. The cells were rinsed, postfixed in 1% osmium tetroxide, and flat-embedded in Epon

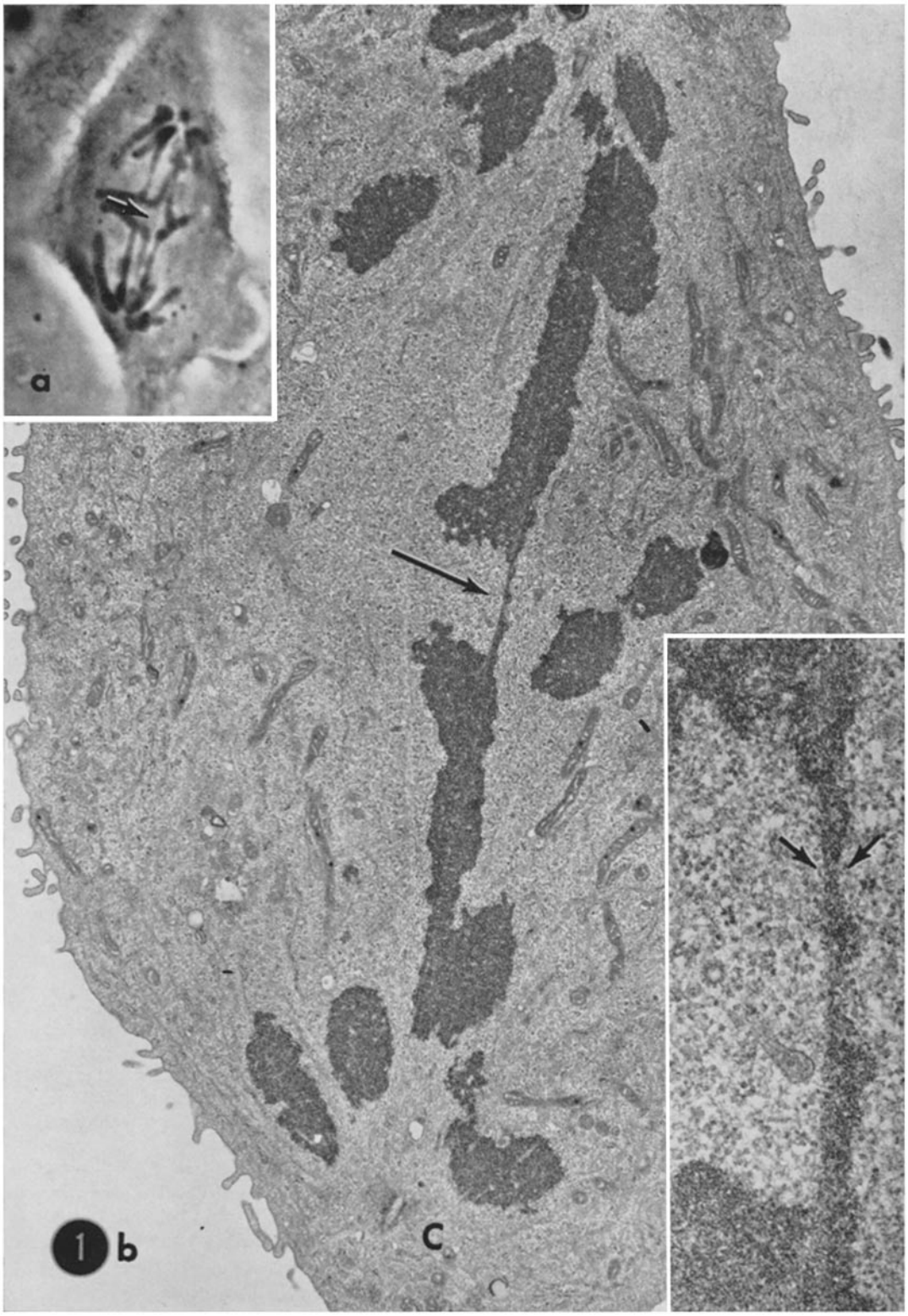

FIGURE 1 *a* Phase micrograph of an anaphase cell with three side-arm bridges. The arrow indicates the bridge which will be examined with the electron microscope.

FIGURE 1 *b* A thin section parallel to the side-arm bridge shown in Fig. 1 *a*. The bridge which was only faintly evident in the phase micrograph is clearly seen in the electron micrograph. The inset shows a higher magnification of the bridge in Fig. 1 *b*. Note the stretched fibrils (arrows). *C*, centriole. *a* × 2.000. *b*, × 15,000. Inset, × 37,400.

812 according to the procedure of Brinkley et al. (1967). After polymerization, the Epon plate was separated from the plastic container and the cells were viewed with a 100 × phase-contrast objective. Anaphase cells were photographed and marked. The marked area was bored out and glued onto an Epon capsule. Serial sections were cut on an LKB Ultratome III with a diamond knife and picked up on collodion-coated slotted grids. The grids were stained in alcoholic uranyl acetate and post-stained in lead citrate. After carbon stabilization, the grids were viewed in an Hitachi HU-11C electron micro-

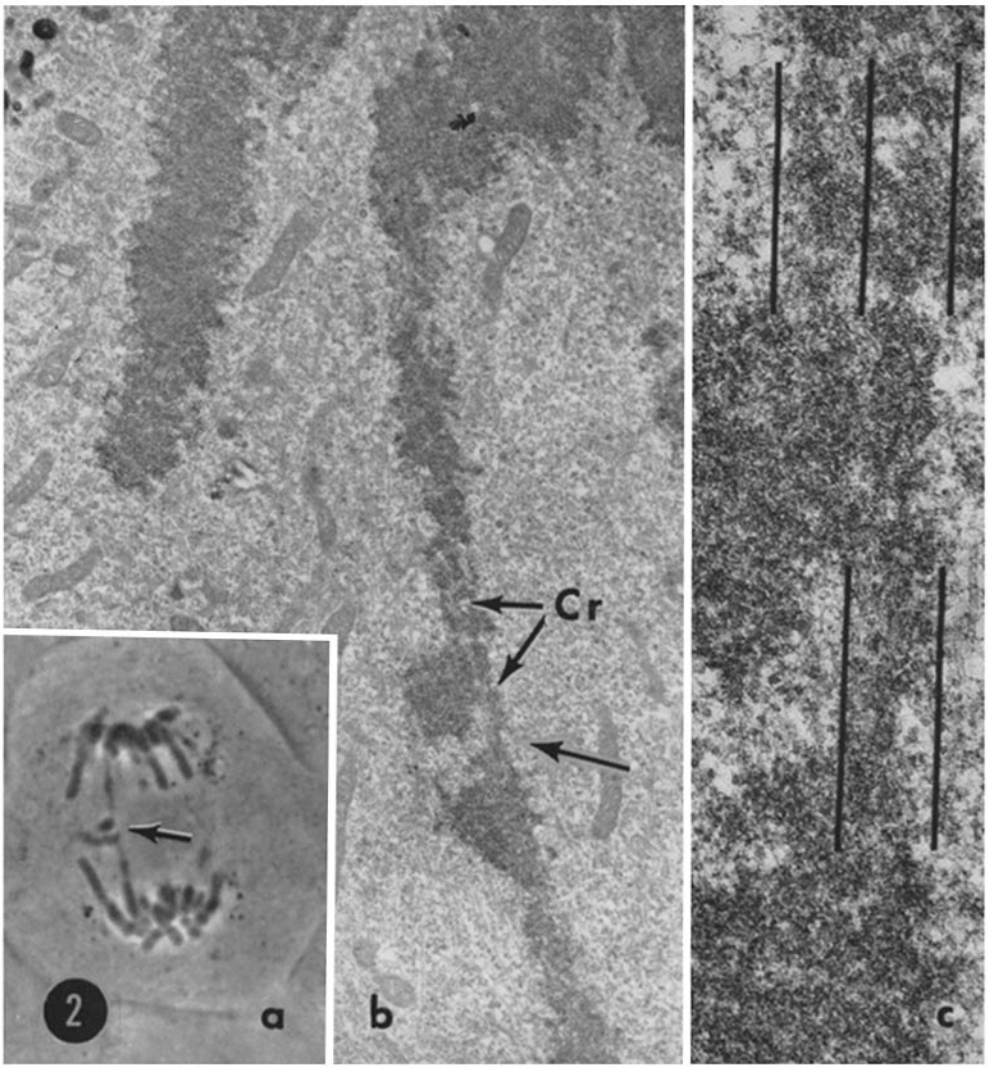

FIGURE 2 *a* Phase micrograph of anaphase with a single side-arm bridge (arrow).

FIGURE 2 *b* An electron micrograph of the bridge shown in Fig. 2 *a* (arrow). Note that the bridge is approximately one-half the diameter of the associated arms. Chromonema-like fibers (*Cr*) are seen in one of the arms.

FIGURE 2 *c* Higher magnification showing details of the bridge. One of the chromonema-like structures in the arm (outlined with three bars) appears to form the bridge (outlined with two bars). *a*, × 2,000. *b*, × 12,000. *c*, × 46,500.

scope operated at 75 kv with a 20-μ objective aperture.

RESULTS AND DISCUSSION

Under the conditions of our experiments, the cells in anaphase at the time of fixation were either in late G_2 or prophase at the time of irradiation. Of 100 irradiated anaphase cells examined with the phase microscope, 39 exhibited side-arm bridges. In unirradiated control cells two anaphase cells out of 30 were scored as having a side-arm bridge.

Fig. 1 *a* shows a phase micrograph of an anaphase cell with three side-arm bridges. The arms undergo an abrupt bend at the site of the bridge. An actual connection, however, is only faintly visible at this resolution (arrow). The same cell is shown in thin section in Fig. 1 *b*. The bridge is observed as a distinct chromosomal "strand" connecting the two large anaphase chromosomes. In this cell, the bridge is considerably less than one-half the diameter of the associated chromosome arms. It is evident, however, in the higher magnification inset that some attenuation of the bridge had occurred due to anaphase stretching. The smallest fibrils contained in the bridge measure 50–80 A in diameter and are also somewhat stretched (arrows). Complete serial sections of this region demonstrated that the bridge consisted of only one unit with a single attachment site on each chromosome arm.

In Fig. 2 *a*, a single side-arm bridge is indicated at the point of the arrow. Note that the bridged region is considerably shorter than in Fig. 1. A bridge was evident between the two arms when a thin section was examined with the electron microscope. In this case, the bridge appears to be approximately one-half to one-third the diameter of the chromosome arms. The uppermost arm in Fig. 2 *b* appears to consist of two or more chromonema-like structures. The fibers making up the bridge seem to be continuous with one of the chromosome components in the upper arm (Fig. 2 *c*).

The fiber forming the bridge is composed of microfibrils with the same dimension (50–80 A) and staining capacity as those which make up the arms and kinetochore region (Brinkley and Stubblefield, 1966; 1969) and nucleolus organizer (Hsu, Brinkley, and Arrighi, 1967). Therefore, it is unlikely that a "matrix" is involved as proposed in earlier studies (Östergren and Wakonig, 1954).

The simplest interpretation of the side-arm bridges shown in this study is that an exchange had taken place involving units smaller than the chromatid. Whether the exchange involves half-chromatids or even smaller units cannot be clearly ascertained by thin-section analyses. The degree of stretching induced by anaphase movement also complicates the interpretation. While these observations are more consistent with a bi- or polyneme chromosome model, alternate interpretations are also possible. Side-arm bridges could also form if each chromatid consisted of a single folded fiber as proposed by DuPraw (1966; see also Kihlman and Hartley, 1967). Thus, a break and exchange at some point between the fibers of each chromatid could produce a side-arm bridge at anaphase. Such an exchange would not necessarily involve a subchromatid fiber. This interpretation appears unlikely if the observations of Peacock (1961), Heddle (1969, in preparation), and Whissel and Heddle (personal communication) are taken into consideration. Their data suggest that irradiation damage induced in G_2 or early prophase is expressed as a chromatid aberration only after an intervening period of DNA replication. Thus, the initial aberration apparently involved a subchromatic unit. The DNA replication data, along with the ultrastructural observations reported in the present study, lend support to the hypothesis that plant and animal chromosomes are multistranded and composed of more than a single DNA complex.

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