The results of the scanning electron microscopy are summarised in the table. Ectoderm cells from the hydroxyurea-treated embryos were different from ectoderm cells from normal embryos, since after 3 min in culture they showed no filopodia (figure 3). After 0.5 h in culture the cells remained featureless and had not flattened onto the glass substrate, and after 3 h the cells had not formed pseudopodia. Endoderm cells from the hydroxyurea-treated embryos were also different from endoderm cells from normal embryos, since they were featureless at all times of culture and did not show pseudopodia at 3 h. Since it was possible that cells were featureless because they were moribund, cell viability was determined by trypan blue exclusion. Cells were prepared as for electron microscopy, and after 3 min and 3 h of culture they were exposed to 1% trypan blue in dissociation medium for 10 min. The results confirmed that at least 87%, and usually 95%, of the cells from both control embryos and hydroxyurea-treated embryos remained viable.

Thus cells from embryos which were prevented by hydroxyurea treatment from undergoing normal morphogenesis did not show the features and changes in vitro shown by cells from normal embryos.

3. Cells cultured in hydroxyurea: An additional experiment was performed to determine whether hydroxyurea had any direct effect on the appearance of *Xenopus* embryonic cells. Ectoderm cells were dissected from normal embryos, and dissociated and cultured in the presence of $10^{-2}$ M hydroxyurea. Cells after 3 min in culture with hydroxyurea (that had been exposed to hydroxyurea during dissociation) were seen to be different from cells from both normal and hydroxyurea-treated embryos - many ectoderm cells showed smooth 'blebs' or 'bulges' (figure 4). After 0.5 h in culture in the presence of hydroxyurea many of the cells were featureless, and a few still showed 'blebs'; after 3 h more of the cells were featureless (table). This finding might suggest that hydroxyurea has 2 effects on cells from *Xenopus* early embryos; a more immediate effect which produces 'blebs', followed by a longer-term effect which prevents the formation of surface features such as filopodia and pseudopodia.

These results suggest that cells from embryos which are prevented from undergoing normal morphogenesis at gastrulation show a deficiency in their properties in vitro, and are compatible with the idea that the study of cells isolated from early embryos and cultured in vitro may increase our understanding of the mechanisms and controls of morphogenesis.

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## Junctions of polytene chromosomes and the inner nuclear membrane[1]

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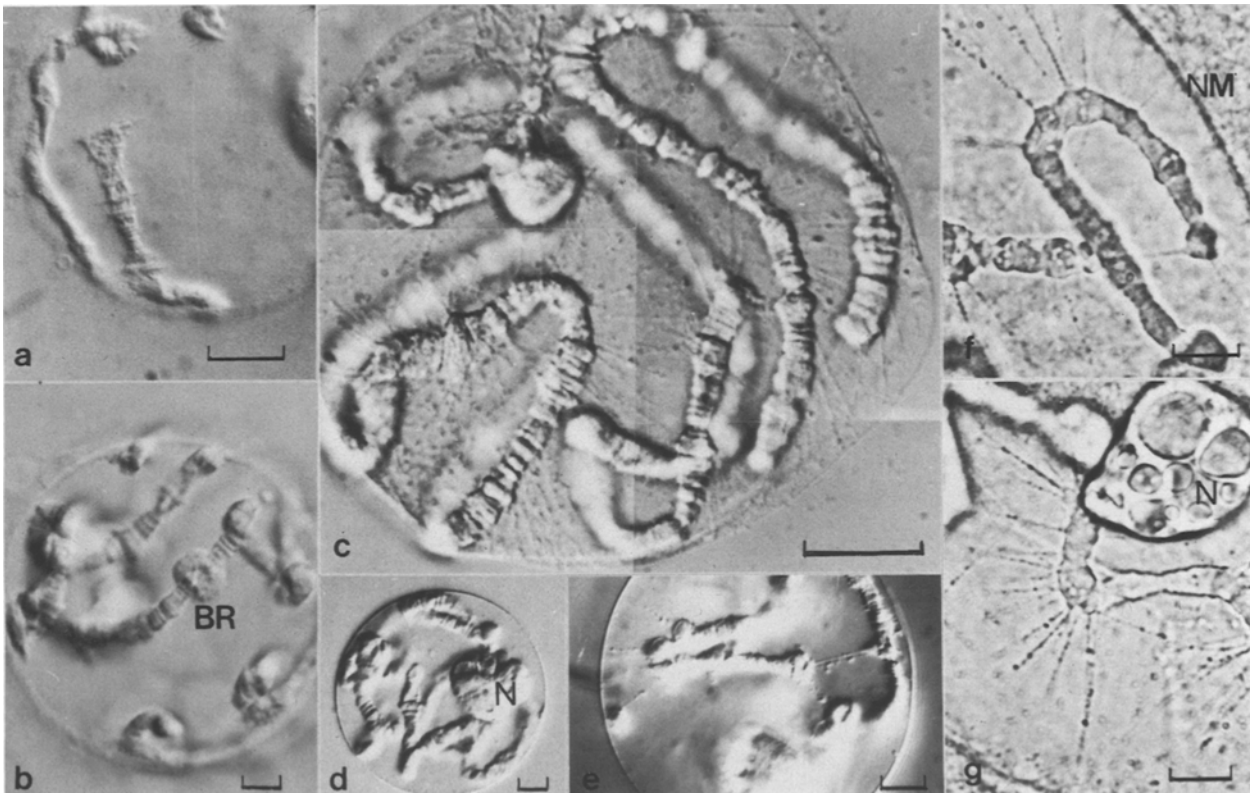
*Summary.* Polytene chromosomes in salivary gland nuclei from chironomid larvae of defined stages are connected along their entire length, by some 100 chromatin fibers, to the inner nuclear membrane.

Chromatin forms structural attachments on the inside of the nuclear envelope, which may serve to hold the interphase chromosomes in fixed positions relative to one another. This was stated for the first time by DuPraw, for honey bee embryonic cells[2]. It is a matter for intensive discussion, that chromosomes are in contact with the nuclear envelope during replication[3]. The first report on salivary gland chromosomes attached to the nuclear envelope was based on the observation of unfixed cells of *Chironomus dorsalis* with phase contrast optics[4]; stereomicroscopical investigation of Feulgen-stained glands yielded more information[5]. In *Chironomus thummi,* strings of connecting material could be determined, with the help of the scanning electron microscope (SEM), in freeze-dried cells which had been forced open[6].

In the present investigation it has been ascertained that salivary gland chromosomes of *Acricotopus lucidus* (raised in the laboratory) and *Chironomus* sp. (collected near Stuttgart) are connected to the inner nuclear membrane, as well as to each other. Light microscopical examination of these connections in isolated polytene nuclei is feasible. Using this method, fibers of 0.2–0.5 µm diameter and up to 45 µm in length have been observed. In addition, gland nuclei from larvae of the last 3 instars have been isolated with a modification of Robert's method using nonionic detergents[7]. Nuclei isolated in such a way show no cytoplasmic contamination (figure, a, c–e); this was assured after SEM-examination of deep-frozen carbon-coated nuclei at magnifications up to 10,000 times[8].

Stretched and beaded fibers between euchromatic and heterochromatic chromosomal segments and the inner nuclear membrane were observed in nuclei during transition from the 3rd to the 4th larval instar. Telomeres are always connected to the nuclear membrane or to each other as described by Bauer[9]; strings deriving from them have the largest diameter of all the fibers, about 0.5 µm. In order to make sure that the fibers are not artefacts produced by the method of isolation[7], single nuclei were excised microsurgically[10]. The nuclei thus obtained are bordered by cytoplasm (figure, b, f, g). This is the case in particular with *Chironomus* nuclei, where the cytoplasm impedes the observation of nuclear structures in an unfixed state. Many fibers tear during prolonged fixation with ethanol-acetic acid (3:1); the micrographs in figure, f–g were taken 30 sec




a–e, Micrographs of unfixed, isolated nuclei, interference contrast. f–g, Isolated nuclei after short fixation, phase contrast. a, b, d, e, Salivary gland nuclei of *Acricotopus lucidus*. a, Early 3rd larval instar. b, d, 4th larval instar. e, Late 3rd larval instar. c, f, g, Polytene nuclei of *Chironomus* sp., transition 3rd–4th larval instar. a, c, d, e, Nuclei isolated with Triton X-100. b, f, g, Nuclei isolated micro-surgically. Notice the fibers connecting the chromosomes with the inner nuclear membrane in c, e, f, g. BR, Balbiani ring; N, nucleolus; NM, nuclear membrane. Scale bars = 10 µm.

after adding a fixative containing carmine. Staining with fluorescent dyes is suitable for morphological examination of unfixed isolated nuclei. With acridine orange the fibers show a red fluorescence which changes during fading to yellowish-green; ethidium bromide causes fluorescence of the fibers, too. The conception that they are chromosomal derivatives is confirmed by enzymatic decomposition; only deoxyribonuclease destroys them (DNase I, Serva); proteinases or ribonuclease do not.

The nodules or beads seem to be stuck on the fibers and do not move to the nuclear membrane; fusing of the beads has not been observed either. The fibers frequently terminate in knobs of 0.5 µm diameter, which rest on the inner nuclear membrane (figure, c); this is evident because isolation of nuclei with Triton X-100 removes the outer nuclear membrane[11]. Some connections of the nuclear membrane with the nucleolus as well as with large puffs (Balbiani rings) were found mostly in isolated nuclei. Those deriving from the nucleolus are smooth, in contradistinction to those from Balbiani rings.

In the early 3rd larval instar any chromosomal section is adjacent to the nuclear membrane (figure, a). Presumably a direct contact exists along the entire chromosomal length during this stage of development. With the increase in the degree of polytenization the length of the chromosomes decreases in comparison with the nuclear volume. This and the altered rigidity of the chromosomes give rise to a changed mechanical situation which does not permit an uninterrupted contact of chromosomes and nuclear membrane in the 3-dimensional system. The appearance of these structures seems to be dependent on changes in the 3-dimensional arrangement during polytenization and growth of nuclear volume. In all probability, during these changes chromatin is drawn out of the polytene chromatid association at distinct points which have a particularly tight contact to the nuclear membrane. Thus, the structures demonstrated are interpreted as relics of an intimate spatial association of polytene chromosomes with the nuclear envelope during the course of replication. It should be noted, however, that the salivary gland nuclei of *Acricotopus lucidus* only pass through 7–8 steps of polytenization at most, therefore nuclei and chromosomes big enough for the observation of lateral chromatin-to-membrane fibers are scarce during transition from the 3rd to the 4th larval instar; in advanced stages of polytenization (more than 2000 C) no fibers apart from interchromosomal and telomere-membrane connections have been observed.

1 Dedicated to Professor Friedrich Mechelke on the occasion of his 60th anniversary.
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