

# THE RABBIT ZYGOTE

## III. Formation of the Blastomere Nucleus

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### ABSTRACT

The formation of the blastomere nucleus was examined in the rabbit zygote with the electron microscope. In late anaphase the chromosomes are bare and vesicles of the smooth endoplasmic reticulum are numerous in the vicinity of the chromosomes. In early telophase individual chromosomes attain their own nuclear envelope and they are called karyomeres. The envelope of the karyomeres contains small gaps within it at several places where the chromatin is exposed to the cytoplasm. Nuclear pores are also observed. In the cytoplasm short annulate lamellae appear adjacent to the karyomeres, and clusters of punctate substance are also present. From early telophase onward the karyomeres extend pseudopod-like structures, called karyopods, which extend toward other karyomeres or karyopods, and consequently fuse together and serve as chromosomal bridges. Eventually all of the karyomeres fuse into a dense nucleus and decondensation of the chromosomes occurs.

### INTRODUCTION

In animal cells during anaphase of mitosis the chromosomes coalesce into an irregular dense mass in each daughter cell and the nuclear envelope is formed around the coalescent mass of chromosomes, the formation of which is completed in telophase (Fawcett, 1966). A deviation from this form of nucleus formation occurs during the formation of the female pronucleus in the lamellibranch, *Mytilus edulis* (Longo and Anderson, 1969) and in the surf clam, *Spisula solidissima* (Longo and Anderson, 1970) and in the sea urchin embryo (Harris, 1961). In the oocytes of both of these species upon completion of second meiotic division chromosomes containing vesicles are formed which in turn fuse and form the female pronucleus. Here we report a similar phenomenon, the formation of karyomeres which fuse by means of karyopods and give rise to the blastomere nucleus in the rabbit embryo.

### MATERIALS AND METHODS

Zygotes were collected between 22 and 24 hr post coitum in order to obtain different stages of first cleavage. The eggs were flushed from the uterine end of the oviducts with warm F10 medium (Ham, 1963). They were transferred immediately into the primary fixative, which consisted of a mixture of 0.25% formaldehyde and 1.0 or 1.25% glutaraldehyde in 0.05 M phosphate buffer, pH 7.4. In some instances 0.1 M *s*-collidine buffer, pH 7.4, was used in place of phosphate. The eggs were fixed for 90 min at room temperature, washed in 0.1 M phosphate buffer for 30 min, and postfixed in ice-cold 1% OsO<sub>4</sub> in 0.1 M *s*-collidine (Bennett and Luft, 1959) buffer, pH 7.48, for 60 min. After osmication the eggs were stained en bloc in 0.5% uranyl acetate in Veronal acetate buffer (Farquhar and Palade, 1965) for 90 min. Subsequently the specimens were rapidly dehydrated in chilled alcohol series of increasing concentration and passed through a series of 2:1, 1:1, 1:2

mixtures of absolute alcohol and Epon 812, respectively, and embedded in Epon 812 (Luft, 1961) of uniform depth of about 1 mm in aluminum boats.

Cubes of Epon, each containing an individual egg, were cut out and cemented on aluminum chucks. 1  $\mu$  sections were stained for light microscopy with toluidine blue in 0.1 M phosphate buffer, pH 7.0. Ribbons of serial thin sections were sectioned with a Du Pont diamond knife on Sorvall MT-2B ultramicrotome. The thin sections were collected on parlodion-coated carbon reinforced copper grids or on Formvar-coated carbon reinforced single hole (1  $\times$  2 mm) copper grids. The sections were briefly stained with aqueous uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) and examined in an AEI-801 electron microscope.

## RESULTS

Two arms of a late anaphase chromosome without the kinetochore region are depicted in Fig. 1 from a dividing rabbit embryo. No membranous structures are adhering to the chromosomes, although some small vesicles are scattered around. In early telophase individual chromosomes attain their own envelope (Figs. 2, 3, 4, and 5), forming karyomeres. In these illustrations either portions or the entire surface of the chromosomes is bordered by two parallel membranes with a space between them. Vesicles which contain flocculent material also become part of the newly forming envelope (Figs. 2, 3, and 6). In these instances that portion of the limiting membrane of a vesicle which adheres to the chromosomes forms the inner membrane of the envelope and the remainder of the limiting membrane of the vesicle forms the outer membrane of the envelope. The cisterna of the vesicle is continuous with the adjacent cisternae of the envelope.

One can observe 600–1200 Å gaps in the newly forming envelope of the karyomeres (Figs. 2, 3, 4, and 5). The gap in the envelope is empty appearing in Fig. 2 (arrows) and chromatin material partially fills up the gap in Figs. 3, 4, and 5 (arrows). Adjacent to these gaps and at other areas of the forming envelope structures are recognizable which bear morphological characteristics of nuclear pores.

Microtubules often remain attached to the chromosomes through the forming envelopes of the karyomeres (Figs. 6 and 7). The cytoplasm immediately surrounding the karyomeres is abundant in small vesicles of the smooth endoplasmic reticulum.

Clusters of punctate substance are also common (Figs. 7 and 9). Single units of annulate lamella are observed at the immediate vicinity of the karyomeres (Figs. 4 and 5). In Fig. 2 the annulated membrane might be continuous with the envelope of the karyomere, but the plane of sectioning does not permit one to ascertain this with certainty.

In early telophase while the karyomeres are attaining their own envelope they extend pseudopod-like structures, hereafter referred to as *karyopods* (Figs. 3, 4, 6, and 8). A karyopod consists of a thin drawn out projection of chromosome bordered by an envelope. At points where a karyopod touches another karyopod or another karyomere they fuse as illustrated at the arrows in Fig. 8 from two consecutive sections. Some karyomeres may have as many as five karyopods extending from them. At the upper portion of Fig. 8 two karyopods have fused with another karyomere and a third karyopod from the upper karyomere of the pair is extending a third karyopod toward the lower karyomere.

Several karyomeres have fused during the formation of the new nucleus as illustrated in Fig. 9. Note that in Figs. 9 *a-c* the karyomeres Nos. 2 and 3 are only touching each other, while karyomere No. 3 has fused with karyomere No. 4 (Figs. 9 *c-e*). Pores are located on the surface of the condensed nucleus and on the karyomeres as well. In the nucleoplasm there are several clear islands and polypous projections of the cytoplasm which usually have membranous structures bordering them. A more advanced stage of karyomere fusion into one condensed nucleus is illustrated in Fig. 10. A total of 20 fused karyomeres have been accounted for in this electron micrograph as determined from serial thin sectioning. Karyopods and narrow chromosomal bridges are still demonstrable at several loci. The chromatin is homogenous and nucleolus formation is not apparent. Nuclear envelope borders almost the entire nucleus except for a few small regions. At these regions one can observe small vesicles in the immediate vicinity of the naked areas.

In the vicinity of the forming nucleus and elsewhere in the cytoplasm various shapes and sizes of paired cisternae are present (Figs. 9 *a-f*) which were discussed in detail by Gulyas (1972). After all of the karyomeres fuse into a compact nucleus the chromosomes rapidly decondense and nucleoli appear (Fig. 11). At first the nucleus is small and has a tortuous configuration, but it rapidly expands assuming a spheroidal shape.

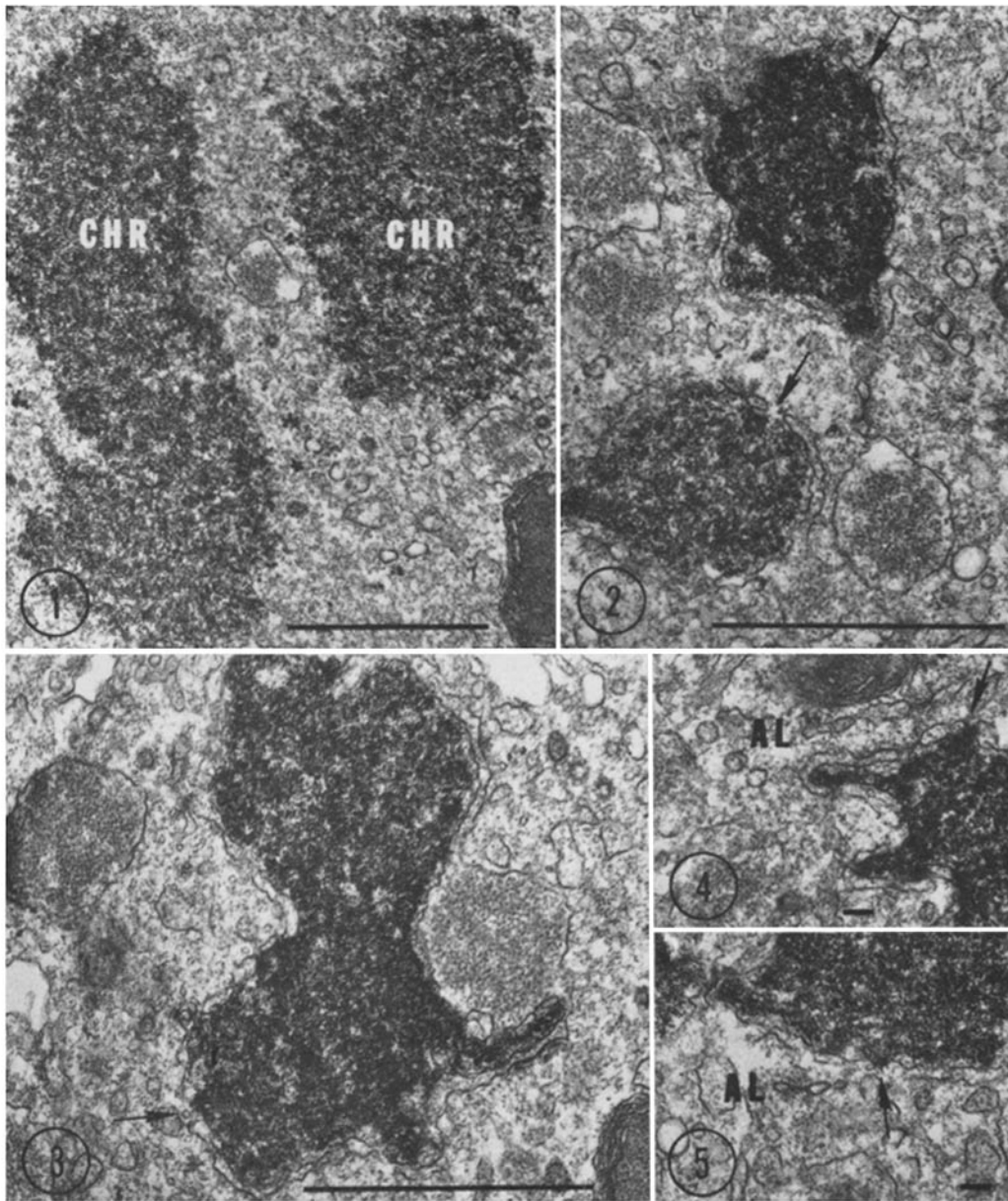
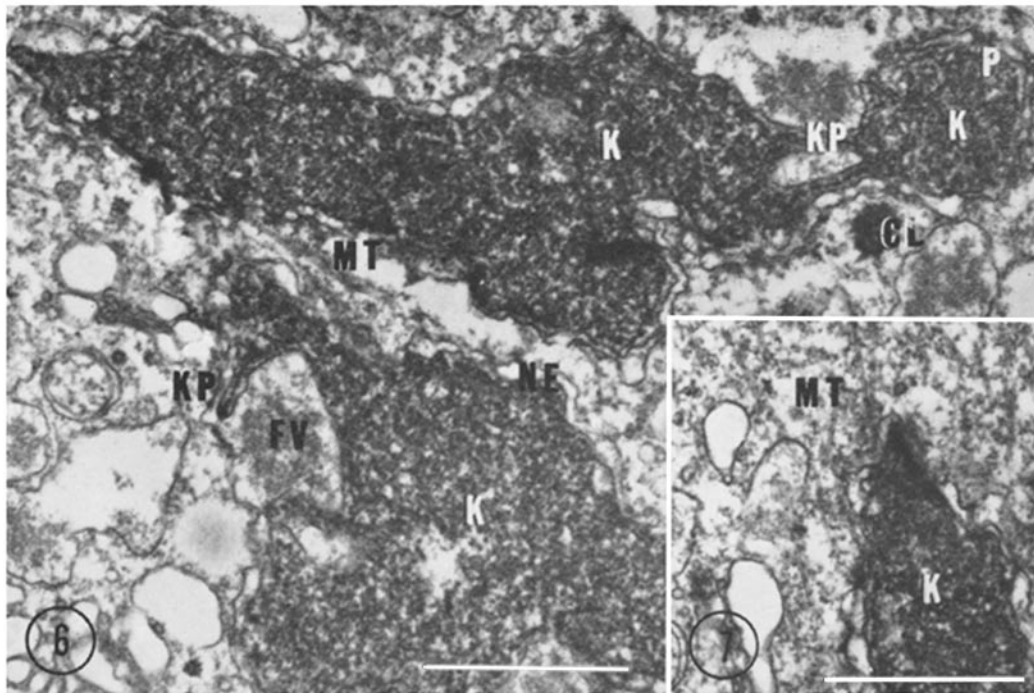


FIGURE 1 Two arms of a late anaphase chromosome (*CHR*) without the kinetochore. Vesicles of the smooth endoplasmic reticulum and flocculent material-containing vesicles are scattered around. Marker is  $1\ \mu$  on all figures unless indicated otherwise.  $\times 27,000$ .

FIGURES 2 and 3 Nuclear envelope forming around individual chromosomes, which are referred to as karyomeres, in early telophase. At arrows gaps are present in the envelope and at other regions nuclear pores are present. Vesicle, containing flocculent material, becomes integral part of the newly forming envelope in Fig. 3. Fig. 2,  $\times 39,500$ ; Fig. 3,  $\times 39,500$ .

FIGURES 4 and 5 Chromatin material partially fills the gap in the nuclear envelope of the karyomeres at arrows, and at other regions pores are present. Short cytoplasmic annulate lamella (*AL*) run parallel with the nuclear envelope. Marker is  $0.1\ \mu$ . Fig. 4,  $\times 39,500$ ; Fig. 5,  $\times 39,500$ .



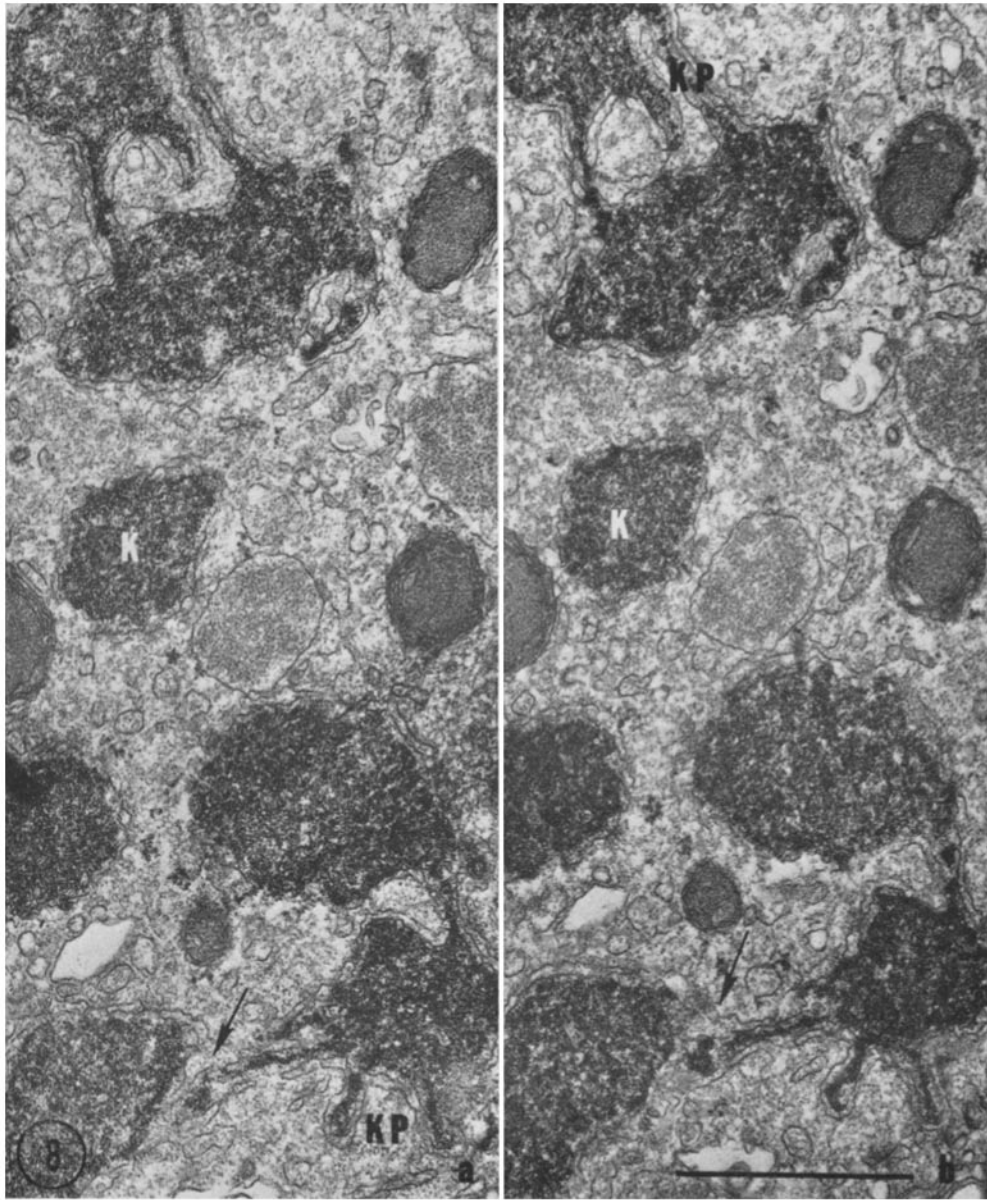
FIGURES 6 and 7 Nuclear envelope (*NE*) is formed around chromosomes in early telophase. Some pores (*P*) are present, karyopods (*KP*) join two karyomeres (*K*) and microtubules (*MT*) penetrate through the *NE*. Vesicles, containing flocculent material, (*FV*) become part of the new *NE*. Fig. 6,  $\times 26,500$ ; Fig. 7,  $\times 26,500$ .

## DISCUSSION

In some cell types the nuclear envelope is retained throughout division and thought to be reutilized during the formation of the nuclear envelope of daughter cell nuclei (Gall, 1964; Porter and Machado, 1960; Szollosi and Calarco, 1970). In the other cell types, single cisterna, resulting from the incomplete breakdown of the nuclear envelope, and paired cisternae, produced by a folding of the nuclear envelope on itself, may be retained throughout cell division. However, neither the single nor the paired cisternae are believed to be reutilized intact in the formation of the new nuclear envelope (Barer et al., 1961; Ito, 1960; Chang and Gibley, 1968). The new nuclear envelope is formed from the endoplasmic reticulum in these cells; however, the possibility cannot be ruled out that cisternae of nuclear envelope origin are converted into smaller fragments of the endoplasmic reticulum and then reutilized in the formation of nuclear envelope.

In the rabbit zygote paired cisternae which are

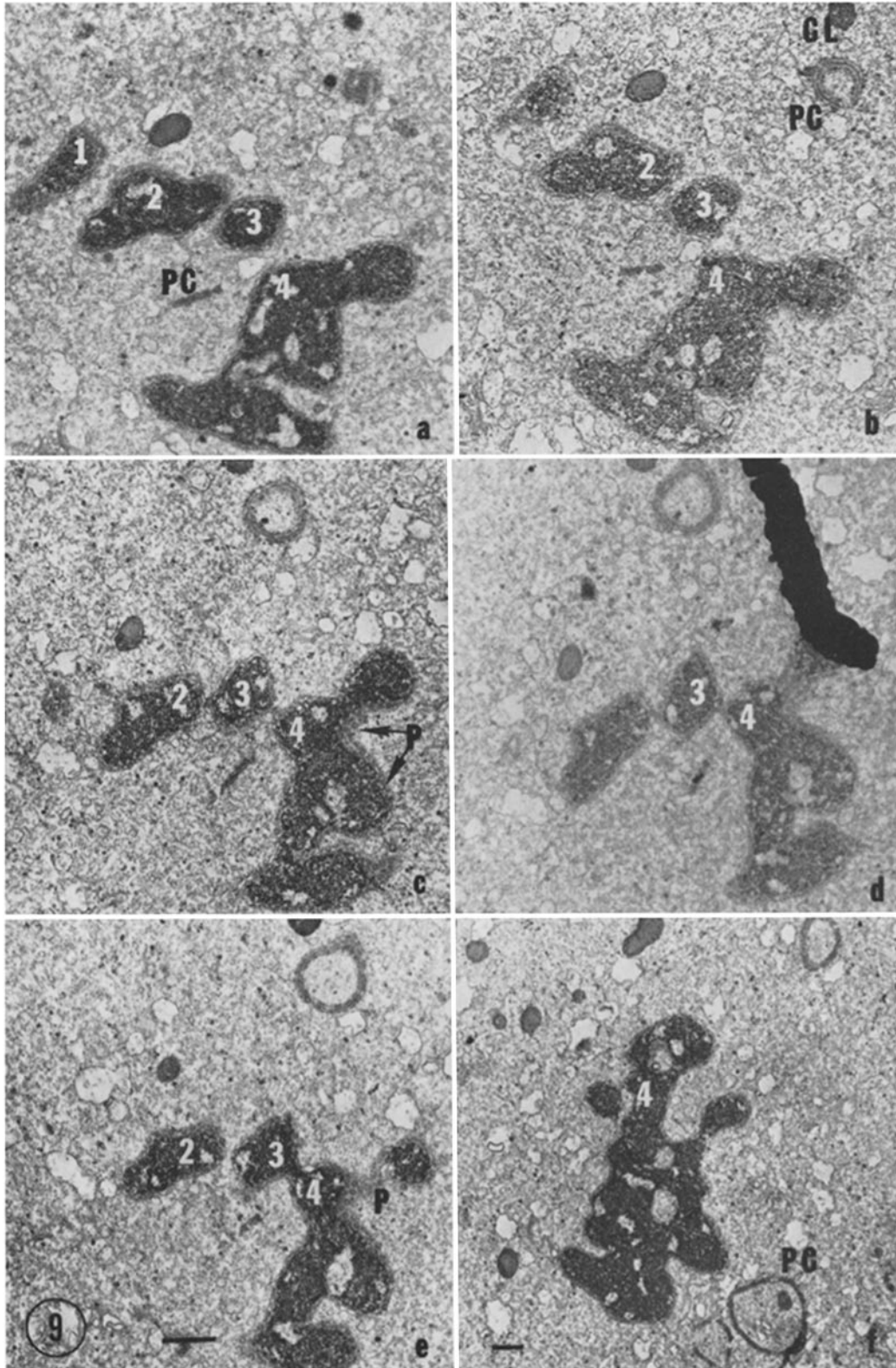
formed from the pronuclear envelopes are observed throughout the entire division (Gulyas, 1972), including early interphase of the daughter cells. However, the presence of annulate lamellae complicates interpretation of the results, since annulate lamellae also lose their pores during division and the remaining cisternae coalesce (Gulyas, 1972), at which time it is no longer easy to distinguish between paired cisternae arising from annulate lamellae and those derived from paired cisternae of the pronuclear envelopes. Coalesced, multilamellar cisternae and beaded, paired, and multilamellar cisternae are the only ones known with some degree of certainty to originate from annulate lamellae (Gulyas, 1972). Regardless of origin, neither paired nor multilamellar cisternae are reutilized intact during the initial stages of nuclear envelope formation of the blastomeres. However, it is conceivable that the paired cisternae are fragmented into small vesicles which in turn are reutilized during nuclear envelope formation, a topic discussed below.



FIGURES 8 *a* and *b* Individual karyomeres (*K*) extend several karyopods (*KP*), some of which fuse with other karyomeres (arrows), such as those illustrated above and below. Few pores are present in the nuclear envelope which may be only partially formed around some chromosomes.  $\times 32,000$ .

In the rabbit embryo the nuclear envelope is reconstructed in telophase around individual chromosomes. Small cisternae of smooth endoplasmic reticulum about the chromosomes, and the nuclear envelope is formed in a manner that is similar to its formation in the rabbit female

pronucleus (Zamboni and Mastroianni, 1966). Vesicles containing flocculent material also become an integral part of the nuclear envelope. At this early stage of nuclear envelope formation pores are not numerous but present. A short time later, during fusion of the karyomeres, pores





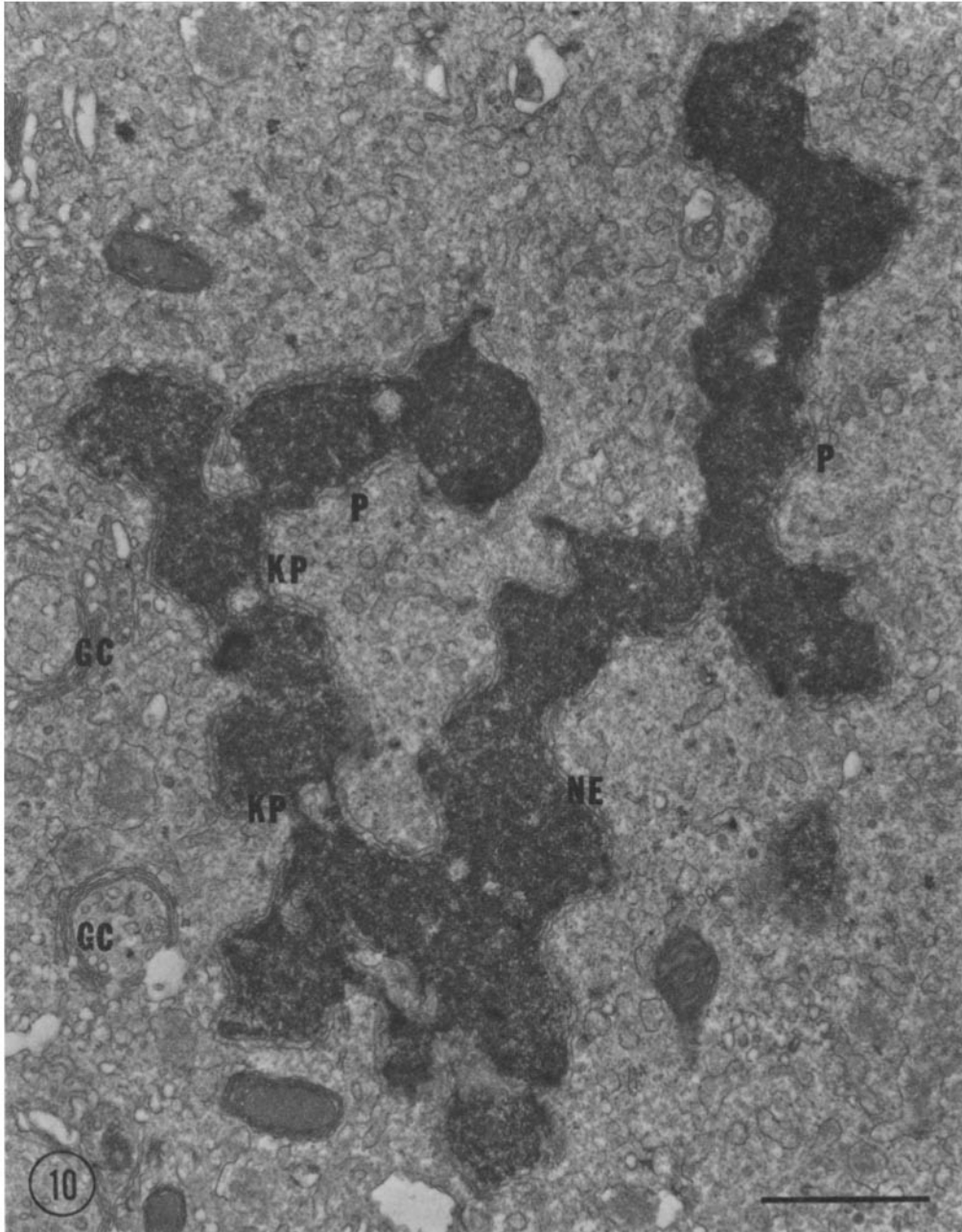


FIGURE 10 A total of 20 karyomeres (determined by reconstruction from serial thin sections) fused into a rather unusually shaped condensed nucleus. Karyopods (*KP*) are still observable at several places; pores (*P*) are present in the nuclear envelope (*NE*). Golgi complex (*GC*).  $\times 23,000$ .

FIGURES 9 *a-f* Reconstruction of fusion of karyomeres into a condensed nucleus. Pores (*P*) are present in the envelopes of the karyomeres. Paired cisternae (*PC*) accompanied by clusters (*CL*) of punctate substance are invariably near the newly forming nucleus. Some karyomeres only touch each other (Nos. 2 and 3) and others, such as No. 3, are fused (*e*) with the blastomere nucleus. Fig. 9 *f* is several sections after Fig. 9 *e*. Figs. 9 *a-e*,  $\times 7800$ ; Fig. 9 *f*,  $\times 5000$ .

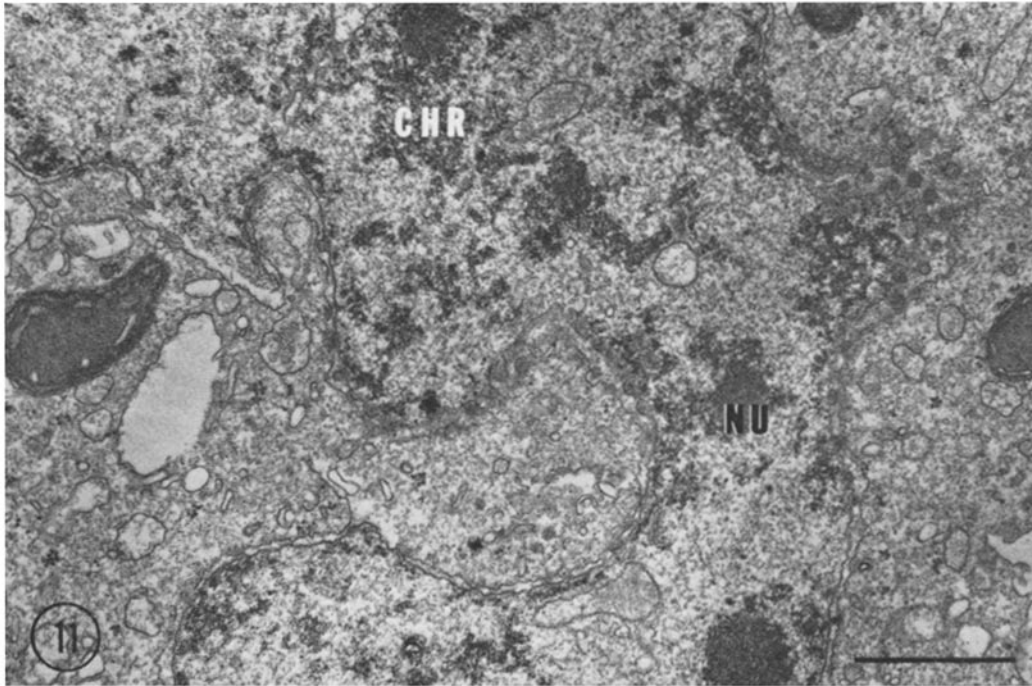


FIGURE 11 Portion of an expanding blastomere nucleus which has a tortuous configuration. Partially condensed chromosomes (*CHR*) are still present and nucleoli (*NU*) appear.  $\times 21,100$ .

become more numerous in the nuclear envelope. The dynamic aspects of pore formation in the envelope of the karyomeres cannot be reconstructed with great certainty from these static observations. However, one postulate can be offered on this topic from the observations presented here. It is conceivable that the small gaps between segments of the new envelope may serve as sites of future nuclear pores. The relationship between the chromatin partially filling these gaps deserves further attention. Furthermore, the significance of the clusters of punctate substance that accompany the forming nucleus and the concomitantly reforming annulate lamellae (Gulyas, 1972) is obscure, but their potential significance cannot be dismissed.

It has been shown previously that the pronuclear envelopes and the annulate lamellae undergo similar alterations during first cleavage (Gulyas, 1972) with regards to disappearance and reappearance of their pores. The appearance of short cytoplasmic annulate lamellae concomitant with pore formation in the nuclear envelope in this study further supports these previous observations.

It is quite evident that in the rabbit embryo the

chromosomes do not clump into one mass before reconstruction of the nuclear envelope. Instead, individual chromosomes precociously attain their own envelope, thus called karyomeres, which fuse with other karyomeres in a rather unique way. The pseudopod-like extensions of the karyomeres, referred to as karyopods, are not mere irregular drawn out or dragging segments of the chromosomes. Firstly, the karyopods are seen in telophase when spindle elongation has ceased. Secondly, the karyopods extend in every direction from the karyomeres, which one would not expect if the extensions were a result of chromosomal dragging. It is proposed that the karyopods form the basis for union of karyomeres. Once the karyopods fuse with other karyomeres they serve as chromosomal bridges between them, eventually leading to complete fusion of the karyomeres into a single, often unusually shaped, condensed nucleus (Figs. 9 and 10). It is not understood what physical properties of the chromosomes give rise to the karyopods or how these recognize another karyomere.

The author is most grateful to Doctors Daniel Szollosi, Griff Ross, and William Tullner for critically re-



viewing the manuscript and offering valuable suggestions in the course of preparing the manuscript.

Received for publication 23 March 1972, and in revised form 31 July 1972.

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