INTRANUCLEAR ANNULATE LAMELLAE IN ASCIDIAN EMBRYOS

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Intranuclear annulate lamellae (IAL) are found in large numbers in oocytes of several species of ascidians (Hsu, 1963; Kessel, 1964, 1965), and there is evidence that these lamellae are attached to the nuclear envelope (Everingham, 1968). The present report describes the occurrence of IAL in ascidian embryos and presents evidence that these IAL also are continuous with the nuclear envelope.

MATERIALS AND METHODS

Adult *Boltenia villosa* (phylum, Chordata; subphylum, Urochorda [Tunicata]; class, Ascidiacea) were collected intertidally. Gametes and embryos were handled according to standard procedures (Fernald, 1959; Costello et al., 1957). Living embryos in gastrula or neurula stages were pipetted into the fixative accompanied by a small amount of seawater. The fixative was either (*a*) 2.7% OsO_4 in approximately 0.2 M bicarbonate buffer, or (*b*) 2% OsO_4 in 0.1 M phosphate buffer. After fixation for 2 hr at 0–4°C, the specimens were rapidly dehydrated in a graded ethanol series, left for 30 min in absolute ethanol, and embedded in Epon (Luft, 1961). Thin sections were stained with lead tartrate (Millonig, 1961) and examined in an RCA 2A electron microscope.

RESULTS AND DISCUSSION

Intranuclear annulate lamellae (IAL) display typical nuclear annuli, as is shown in longitudinal section in Figs. 3–5. It is important that IAL be distinguished from profiles of indented portions of the nuclear envelope which surround cytoplasmic embayments (*CE*, Fig. 1). Profiles of true IAL may be recognized by their noncircular nature and by their lack of associated cytoplasm. The identification may be confirmed by serial sections. Figs. 2 and 3 represent adjacent sections in one such series. In another series, one IAL (Fig. 5) spans seven consecutive sections; this lamella is therefore at least 0.5 μ wide, and possibly considerably wider.

Early ascidian embryos are composed of six types of cells: ectodermal, endodermal, neural, chordal, myoblast, and mesenchymal (see Reverberi, 1961); IAL were observed in each type. The IAL of embryonic cells are much less numerous than those of large oocytes, and they rarely measure more than 1 μ in length. The IAL in embryonic nuclei frequently (Figs. 1–3, 5), but not invariably (Fig. 6), lie near a patch of condensed chromatin (*CC*). In both embryos and oocytes, the IAL are located near the nuclear envelope. Direct continuity of an IAL and the nuclear envelope is illustrated in Figs. 4 and 6. One can see that the inner membrane of the nuclear envelope is invaginated and becomes continuous with the edge of an intranuclear annulus (*ANN*, Fig. 6).

The IAL of these embryonic nuclei may represent remnants of the nuclear envelopes of chromosomal vesicles (karyomeres) (Harris, 1961; Thomas, 1964), which have been entrapped in the nucleus during telophase and have persisted into interphase. Such an interpretation, however, cannot apply to the IAL of ascidian oocytes, or to the IAL present in the male pronuclei of penetrated mammalian ova (see below).

This note is, to the author's knowledge, the first published report of the occurrence of IAL in interphase nuclei of embryonic cells. However, similar structures have been observed in the pronucleus of the unfertilized sand dollar egg (Merriam, 1959), as well as in the pronuclei of penetrated ova of several mammalian species including man (Zamboni et al., 1966), the hamster

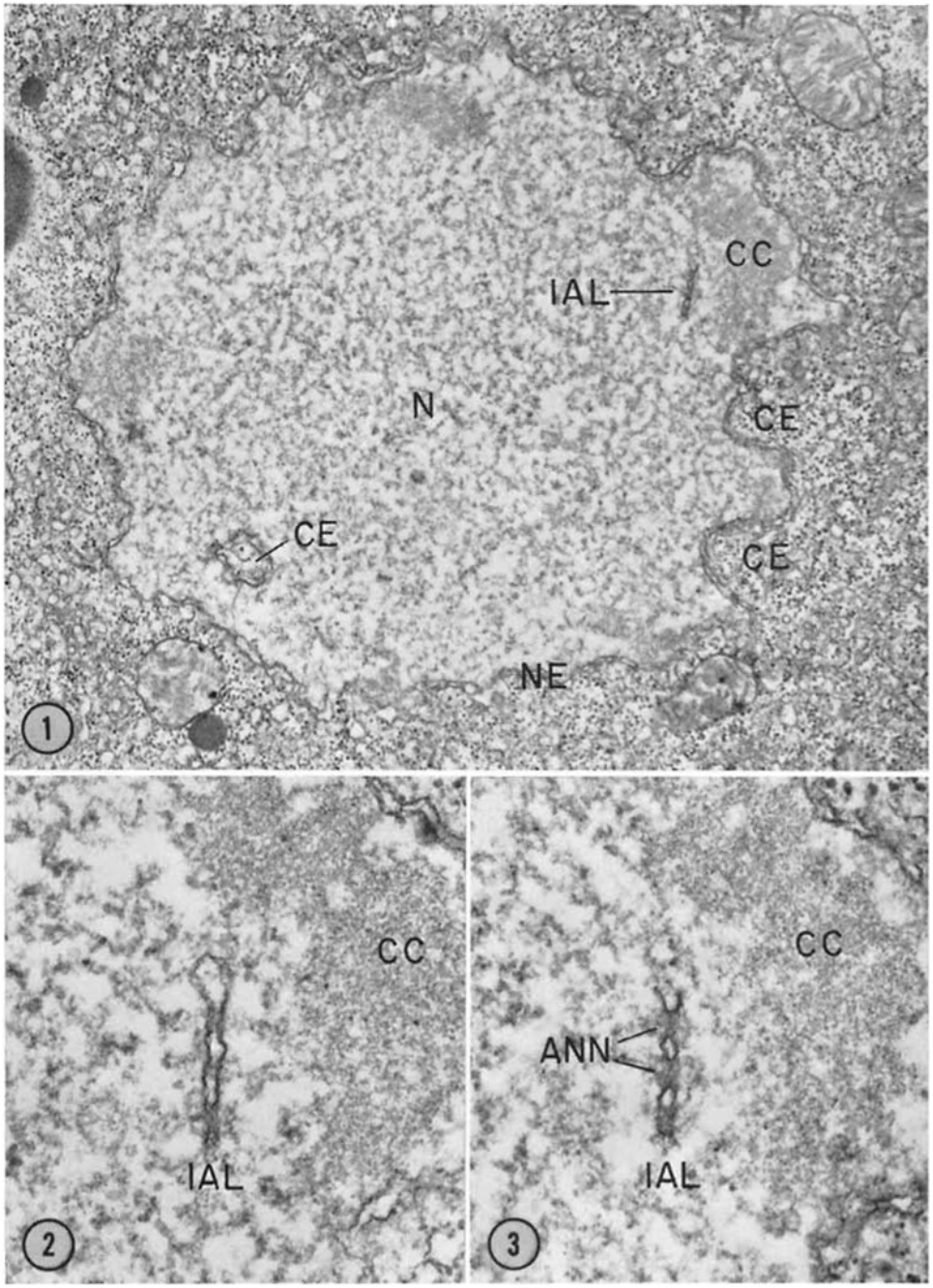
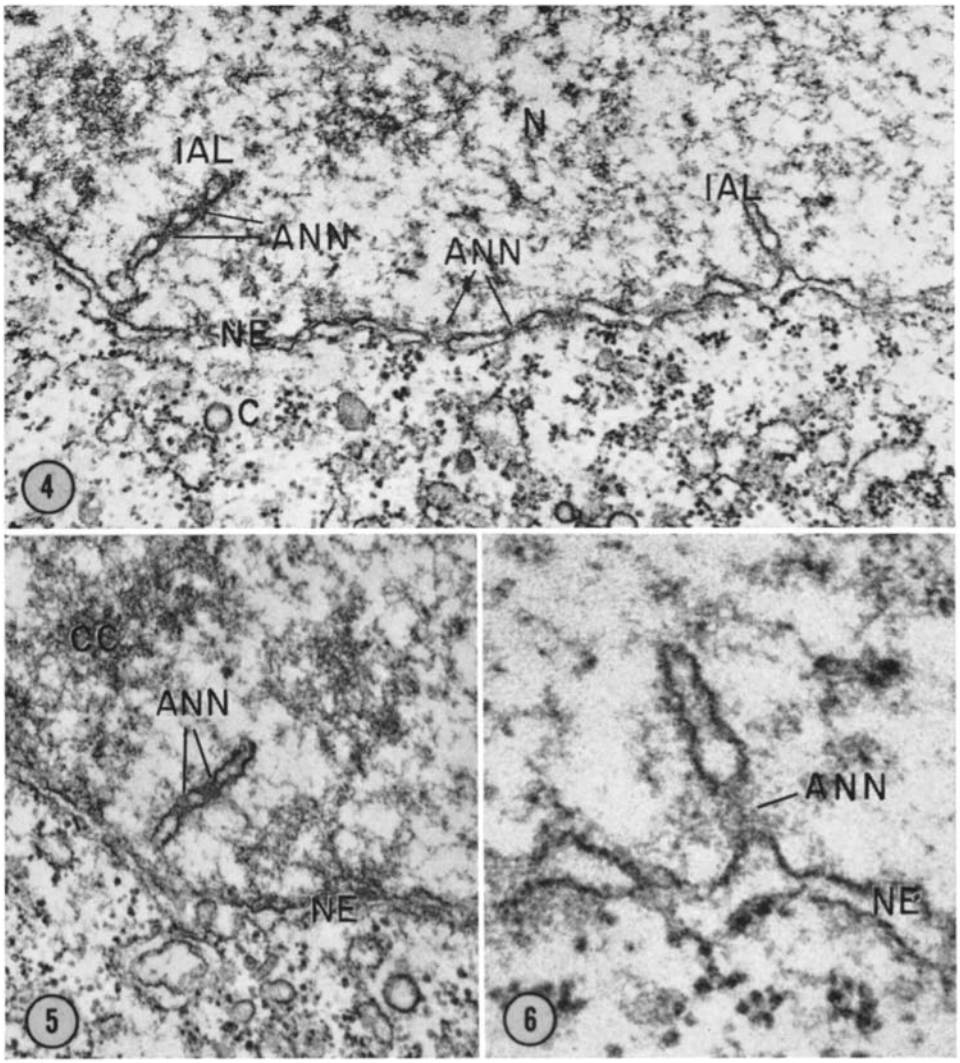


FIGURE 1 Nucleus (*N*) and paranuclear structures. An intranuclear annulate lamella (*IAL*) is seen near a patch of condensed chromatin (*CC*). The nuclear envelope (*NE*) is seen to surround the nucleoplasm and to line several cytoplasmic embayments (*CE*) which indent the surface of the nucleus. Presumptive neural cell of a neurula-stage embryo. × 15,000.

FIGURE 2 This micrograph represents a section adjacent to the one depicted in Figs. 1 and 3. *IAL*, intranuclear annulate lamella; *CC*, condensed chromatin. × 41,000.

FIGURE 3 From the same section as Fig. 1. The *IAL* profile shows two annuli (*ANN*) sectioned longitudinally. *CC*, condensed chromatin. × 41,000.



Figs. 4–6, intranuclear annulate lamellae (*IAL*) in a presumptive neural cell of an embryo in transition between the gastrula and neurula stages. *N*, nucleus; *C*, cytoplasm; *NE*, nuclear envelope. From a series of seven adjacent sections.

FIGURE 4 Two *IAL* associated with the nuclear envelope. Longitudinally-sectioned annuli (*ANN*) are seen in both *IAL* and in the nuclear envelope. × 34,000.

FIGURE 5 This *IAL* corresponds to the one on the left in Fig. 4 and is separated from it by an intervening section. The *IAL* appears to be surrounded by patches of condensed chromatin (*CC*). × 34,000.

FIGURE 6 The annulus (*ANN*) of the *IAL* is continuous with the inner membrane of the nuclear envelope (*NE*). This figure is a photographic enlargement of the *IAL* on the right in Fig. 4. × 88,000.

(Szollosi, D. G. Personal communication.), and possibly the rabbit (Zamboni and Mastroianni, 1966). In man and in hamster, both male and female pronuclei contain IAL. In several embryonic stages of the mouse, IAL are observed to be attached to the nuclear envelope (Hillman, N. Personal communication. Szollosi, D. G. Personal communication.). IAL have been observed in

polar bodies of *Boltenia villosa* (Everingham, J. W. Unpublished observations.). In addition, Zamboni et al. (1966) reported the occurrence in human polar bodies of intranuclear lamellae whose annulate nature they could not identify with certainty. Bucciarelli (1966) described the presence, in a Rous sarcoma cell, of intranuclear lamellae which are nonannulate, but which may be related to the inner membrane of the nuclear envelope.

There may be good reasons for the paucity of published observations of intranuclear annulate lamellae. IAL may appear only briefly at a particular stage in a developmental sequence, and thus may be little regarded and unreported by microscopists who have observed them. They may have escaped observation in the past because of their liability to disruption by preparative procedures (see Everingham, 1968), and the greater frequency of recent reports may reflect improvements in preparative technique. These considerations suggest that intranuclear annulate lamellae may be more widely distributed in the natural world than has been recognized heretofore.

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Note Added in Proof: N. W. Hillman's studies of early mouse embryos were presented before The American Society for Cell Biology and have been published in abstract form: 1967. *J. Cell Biol.* **35**:113A.

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