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Oogenesis

A study of the determination of spatial pattern in embryos clearly has to start long before fertilisation, in particular to find out how much pattern has been laid down during the development of the egg within the mother. This stage, oogenesis, has been extensively studied in recent years so that we now know quite a lot about the acquisition of organelles and molecules which will be used later in the embryo.

Brief background information about these processes will be given here. Evidence for patterned arrangements of materials within oocytes will be considered next. Finally, we will ask whether such patterns imply that the parts of the oocyte are already determined, i.e. have they specialised so that they are only capable of forming certain parts of the embryo?

The events of oogenesis

Oogenesis, of course, includes the division of the primordial germ cells to produce successive generations of oogonia, their eventual transformation to oocytes and the meiotic divisions of the oocytes. Most of the obvious preparation of the egg occurs in the prophase of the first meiotic division during which the primary oocyte grows, mainly by an increase in its stores of yolk, and there may be obvious signs of activity in the nucleus and elsewhere. The volumes of animal eggs are usually orders of magnitude greater than those of somatic cells, and the nucleus of the oocyte is also enlarged and commonly called the germinal vesicle. Figure 1.1 gives a good impression of the increasing complexity of the oocyte and its investments during six stages of oocyte development in the frog *Xenopus laevis*. Massive numbers of organelles accumulate in temporal and spatial patterns which show at least some specificity. Some will have obvious roles in embryonic development, e.g. as sources of protein and energy (yolk) or in the release of energy (mitochondria), while the roles of other organelles characteristic of oocytes, such as cortical granules and annulate lamellae, will be discussed later (see especially pages 7 and 31–2). In most cases the accumulation of yolk (vitellogenesis) is a relatively late

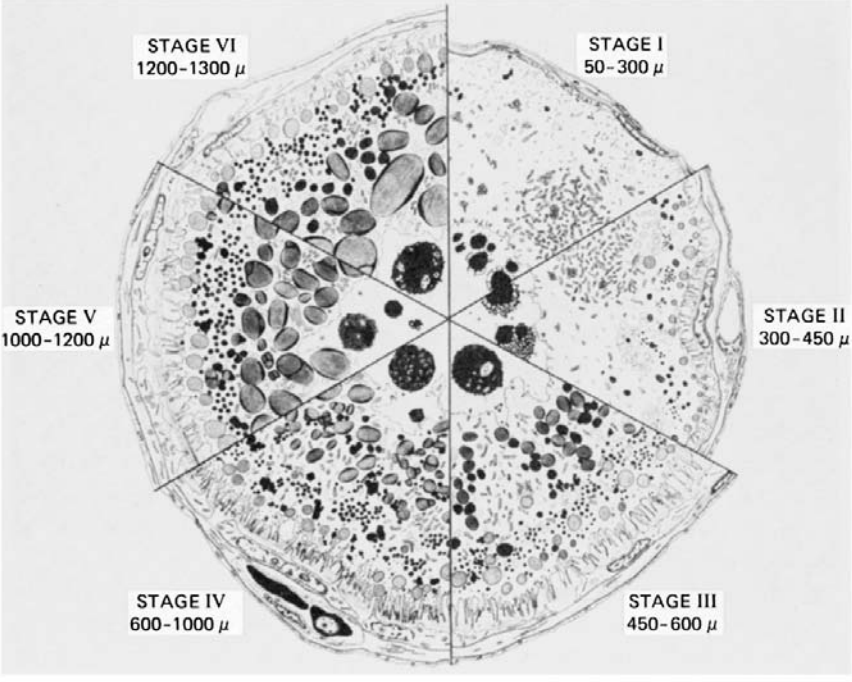

Fig. 1.1. A diagrammatic representation of six stages in *Xenopus* oocyte development, not drawn to scale. (From Dumont, 1972, where further details may be obtained.)

and rapid event (in *Xenopus* mainly at stage IV) following a longer previtellogenic stage.

The genome in the primary oocyte has already been replicated in preparation for the meiotic divisions and so there is a tetraploid (4*n*) amount of DNA present in the nucleus. At some stage of first meiotic prophase (often at diplotene), the chromosomes commonly take on the lampbrush form, in which loops of chromatin are seen to project from the chromosome axis. RNA is synthesised rapidly there (Gall & Callan, 1962), with RNA polymerase molecules packed far more densely together than is usual on somatic cell DNA (Miller & Bakken, 1972). About 5% of the genome is present in the lampbrush loops of the newt *Triturus* (Callan, 1963), and an enormous amount of genetic information is transcribed. As in most cells, much is lost during processing within the nucleus, but even so a variety of animal eggs retain RNAs with a complexity of some tens of millions of nucleotides (Davidson, 1986). The *Xenopus* egg is supplied with some 20,000 different poly(A)$^+$RNAs, for most of which there are about 10^6 copies (Perlman & Rosbash, 1978).

Among the messenger RNAs transcribed in the oocyte, several have

been identified which will have important roles in development. These include mRNAs for histones (Gross *et al.*, 1973), tubulin (Raff *et al.*, 1972) and ribonucleotide reductase (Noronha, Sheys & Buchanan, 1972; see too Standart *et al.*, 1985). Much of the protein synthesis occurring in early embryos is supported by mRNA derived from the oocyte – until blastula stages in sea urchins (Gross, 1964) and *Xenopus* (Crippa, Davidson & Mirsky, 1967), and even in tadpoles of the rapidly developing tree frog *Engystomops* (Hough *et al.*, 1973) and perhaps ascidians (Lambert, 1971). Many specific mRNAs are present in the maternal pool and in the embryo but are absent from adult organs, and it has been proposed that these code for 'morphogenesis proteins' needed to construct an early embryo (Hough-Evans *et al.*, 1977). The lampbrush form could allow this set to be built up as quickly as possible using the full $4n$ genome.

Perhaps the most seductive evidence for this interpretation of lampbrush chromosomes is the existence of exceptions which seem to prove the rule. These are the meroistic ovaries of many insects where oocytes are syncytially connected to sister cells acting as nurse cells. Figure 1.2 shows two kinds of meroistic development and compares them with the simpler panoistic system. Where nurse cells are present, the oocyte chromosomes appear relatively inactive and certainly no lampbrush stage is seen. The nurse cell nuclei, on the other hand, produce RNA very fast indeed and there is histochemical (Bonhag, 1955) and autoradiographic evidence (Bier, Kunz & Ribbert, 1967) for massive transfers of RNA to the oocyte (see Fig. 1.2). This seems to include at least the bulk of the oocyte mRNAs (Winter, 1974; Paglia, Berry & Kastern, 1976; Capco & Jeffery, 1979). In such cases there are many nuclei transcribing genomic information for the oocyte and each of these nurse cell nuclei may be highly polyploid; and it is in just such cases that oogenesis is particularly rapid. The lampbrush form and the meroistic system therefore seem to be alternative mechanisms to produce the same end – the rapid transcription of the genomic information for early development.

There are, in fact, other cases which indicate the high 'cost', in terms of information, of gametogenesis and early development. In some embryos many chromosomes are actually eliminated early from all somatic lineages, only the germ-line retaining the full chromosome complement (e.g. *Ascaris*: Boveri, 1887; gall-midges: Kahle, 1908). In the oocytes of one gall-midge the 'extra' chromosomes are particularly active in RNA synthesis (Kunz, Trepte & Bier, 1970). Similarly there is evidence that both X chromosomes are active in the oocytes of mammals (Epstein, 1969), while one is soon inactivated in all somatic lineages of female embryos (see Lyon, 1972).

One problem for the views summarised above arises from the dynamics

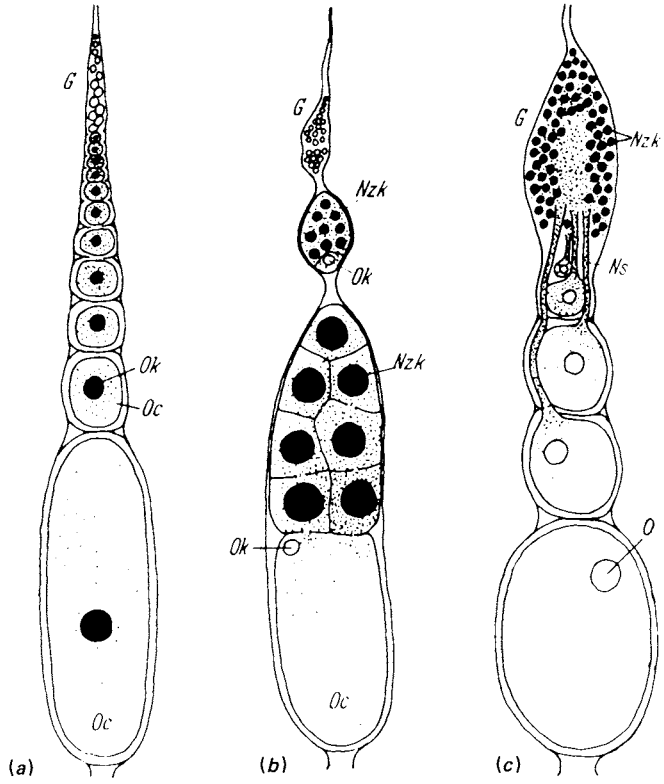

Fig. 1.2. Three types of insect ovary: (*a*) panoistic; (*b*) meroistic polytrophic; (*c*) meroistic telotrophic. Nuclei very active in RNA synthesis are shown black and those relatively inert white; RNA transferred to the cytoplasm is shown as black dots. G, germarium; Oc, oocyte; Nzk, nurse cell nucleus; Ns, trophic cords. (After Bier, 1967.)

of transcript accumulation. Lampbrush loops are present at all six of Dumont's stages in *Xenopus* (see Davidson, 1986) and the mRNAs present at all stages code for the same proteins (Darnbrough & Ford, 1976). Yet, by the start of vitellogenesis (in Stage II), the final quantity of oocyte poly(A)$^+$RNA, and of a large number of specific RNA sequences, has already been accumulated (Golden, Schafer & Rosbash, 1980). Moreover, these early transcripts have half-lives estimated as 2–3 years (Ford, Mathieson & Rosbash, 1977), which should allow them to persist into the embryo. In these circumstances it is difficult to understand why the lampbrush form is retained after Stage II, but both synthesis and degradation rates for poly(A)$^+$RNAs seem to increase continuously through Stage III (when the most extended lampbrush loops are seen) to reach their maximum in full-grown oocytes (Dolecki & Smith, 1979).

There are a few instances of stage-specific transcription in lampbrush loops (Macgregor & Andrews, 1977), but much greater qualitative changes in the mRNA population are seen in sea urchins (Hough-Evans *et al.*, 1979) where true lampbrush matrices do not seem to exist (see Davidson, 1986).

Still more fundamental is the problem that much of the poly(A)$^+$RNA produced for the sea urchin or amphibian egg is untranslatable (Posakony *et al.*, 1981; Thomas *et al.*, 1981). In many ways the maternal RNA molecules resemble nuclear RNAs in other cells, and they may require further processing in the embryo before being translated or may act rather to regulate the expression of other genes in development (Thomas *et al.*, 1981; Shiokawa, 1983). It is also possible, as pointed out by Colman (1983), that the high transcriptional activity of oocytes leads to unregulated synthesis or incorrect splicing of transcripts. It is relevant here to note that concentrations of the small nuclear RNAs, thought to be involved in mRNA processing, apparently fall once their synthesis stops in the young oocyte (Fritz *et al.*, 1984).

Informational RNAs may seem likely candidates to confer spatial organisation on the oocyte, but development of course also requires relatively massive amounts of other classes of RNA. There are, in fact, efficient and specialised methods of providing oocytes with the RNA species in ribosomes and with transfer RNA. To provide the huge amounts of ribosomes required by large oocytes, the major genes (rDNA) present at the nucleolus organisers are replicated – the first known and probably most dramatic case of specific gene amplification – providing thousands of copies of the nucleolar core ring in amphibian oocytes (Painter & Taylor, 1942; Gall, 1968; Perkowska, Macgregor & Birnstiel, 1968). Even some smaller oocytes, including mammalian ones, show a more limited amplification (Brown & Dawid, 1968; Wolgemuth, Jagiello & Henderson, 1980). All of this rDNA can later be transcribed repeatedly as rRNAs (Miller, 1973), which are assembled with 5S RNA and ribosomal proteins into ribosomes. This can occur with such rapidity that the nucleoli bleb out of the nucleus. The oocytes of fish and amphibians show another highly unusual feature in the system used to synthesise the 5S RNA of ribosomes (Ford, 1971; Wegnez, Monier & Denis, 1972; Mazabraud, Wegnez & Denis, 1975; Denis *et al.*, 1980). This is encoded by a particular 5S gene present in multiple copies in the genome but only activated in oocytes. Most 5S RNA is produced in early oogenesis and is stored in 7S particles or 42S complexes (which also contain transfer RNAs) until rRNAs are produced.

Despite the fact that mRNAs and translational machinery are present in abundance in oocytes, only a very low proportion of the ribosomes (Woodland, 1974; Davis, 1982) or the mRNAs (Rosbash & Ford, 1974; Paglia *et al.*, 1976) is usually found to be engaged in protein synthesis

there. Translation appears to be restricted in a variety of ways according to the species and even the stage of oogenesis. Most of the ribosomes in the oocytes of lizards (Taddei *et al.*, 1973) and some mammals (Burkholder, Comings & Okada, 1971) are found in large crystalline aggregates or lattices which are probably an inactive storage form. 'Free' mRNAs are complexed with proteins in mRNPs, and in the eggs of sea urchins (Jenkins *et al.*, 1978) and the chironomid midge *Smittia* (Jäckle, 1980*a*) there is evidence that some of these proteins 'mask' the mRNA preventing its translation. In *Drosophila* there may be both a general limitation of the translational apparatus (Goldstein, 1978) and a selective exclusion of some mRNA species from polysomes (Mermod, Schatz & Crippa, 1980). As *Xenopus* oocytes reach full size, the block seems to be switched from mRNPs (Richter & Smith, 1984; Taylor, Johnson & Smith, 1985) to other components required for translation (Laskey *et al.*, 1977), possibly a specific initiation factor (Audet, Goodchild & Richter, 1987). mRNAs in the tobacco hornworm oocyte apparently lack a 5′methylated cap structure which would make them untranslatable (Kastern & Berry, 1976).

Among the protein species synthesised in oocytes are many which will be required in large amounts by early embryos. As a preparation for the rapid nuclear multiplication of cleavage at least some oocytes form large stores of DNA polymerases (Tato, Gandini & Tocchini-Valentini, 1974), histones (Adamson & Woodland, 1974; Woodland & Adamson, 1977), other proteins required for chromatin assembly (Laskey *et al.*, 1978; Kleinschmidt *et al.*, 1983) and tubulin needed in the mitotic apparatus and elsewhere (Raff *et al.*, 1971; Miller & Epel, 1973; Bibring & Baxendall, 1977). Another cytoskeletal protein formed is actin (Ruddell & Jacobs-Lorena, 1984). At the time of ribosomal assembly in *Xenopus*, more than 30% of the proteins synthesised are ribosomal proteins (Hallberg & Smith, 1975). Large amounts of RNA polymerases are also present in oocytes (Wassarman, Hollinger & Smith, 1972; Roeder, 1974; Kastern, Underberg & Berry, 1981), despite the fact that transcription rates are, at first, low in embryos. Levels of free and organelle-bound enzymes rise through oogenesis (Miller & Epel, 1973), suggesting again a preparation for extensive metabolic activity in the embryo. Even fibronectin, which has a primarily extracellular role from blastula stages in amphibians, is already formed in oocytes (Darribère *et al.*, 1984).

A major event of oogenesis is the accumulation of the protein yolk. The proteins are normally formed elsewhere in the maternal body and can be taken up from blood serum even against a concentration gradient (Knight & Schechtman, 1954; Telfer, 1954): in vertebrates the liver synthesises these proteins. In a variety of animal oocytes, uptake occurs into coated vesicles (Anderson, 1964; Roth & Porter, 1964: another phenomenon first described in oocytes). The proteins are then packed in

a crystalline array in yolk platelets (see Wallace, 1972). In some cases, protein yolk seems formed at least partly in the oocyte (crayfish: Ganion & Kessel, 1972), nurse cells (some polychaetes: Emanuelsson & Anehus, 1985) or follicle cells (*Octopus*: O'Dor & Wells, 1973; *Drosophila*: Brennan *et al.*, 1982). Other storage materials such as glycogen granules and lipid droplets are commonly classified as yolk (see Raven, 1961) but have received less study. Eggs of most placental mammals are often described as lacking yolk, but even in these there is evidence (discussed by Schultz, Letourneau & Wassarman, 1979) for uptake of exogenous proteins by oocytes and breakdown of proteins in early development.

The accumulation of yolk has many effects on development beyond the supply of respiratory substrates and precursor materials. The extent of yolk is often the major factor determining egg size which ranges from less than 100 μm diameter in yolk-poor eggs up to many centimetres in some birds. Where development requires interaction of the areas of the embryo, this has great implications for the distances over which interaction must occur, a topic to which we shall repeatedly have to return. Likewise, we shall many times see effects of yolk upon cleavage pattern. Suggestions of more specific developmental roles for yolk (discussed by Counce, 1973) seem less likely, however, in view of the development of yolkless embryo fragments (Hadorn & Müller, 1974: see also p. 184).

Oocytes also contain a full range of other organelles. There are very large numbers of mitochondria (e.g. see Marinos & Billett, 1981), which will be involved in energy release in the embryo. Other organelles characteristic of oocytes are multivesicular bodies, annulate lamellae and cortical granules. Annulate lamellae are stacked membraneous complexes with an organisation similar to the nuclear membrane. For example, they possess 70 nm diameter pores (see review of Kessel, 1983). Kessel has long suggested that the role of annulate lamellae is to activate stored genetic information very much in the same way as polysomes can assemble at normal nuclear pores. Cortical granules (see review of Guraya, 1982) usually form in late previtellogenesis as Golgi derivatives and move to positions below the plasma membrane: their role is considered in the next chapter (p. 31). Oocytes also have a cytoskeleton which can be quite complex: in amphibian oocytes, for instance, there are large amounts of microtubule protein (Pestell, 1975), actin-containing microfilaments (Franke *et al.*, 1976) and several kinds of intermediate filaments (Franz *et al.*, 1983; Godsave *et al.*, 1984*a*,*b*). Potentially, a cytoskeleton could act as a framework for the localisation of developmental information, and we shall have repeated cause to consider this possibility.

In meroistic ovaries the nurse cells supply many organelles as well as RNA to the oocyte. Often the nurse cells are almost totally absorbed into the oocyte by the time oogenesis is complete. The materials are trans-

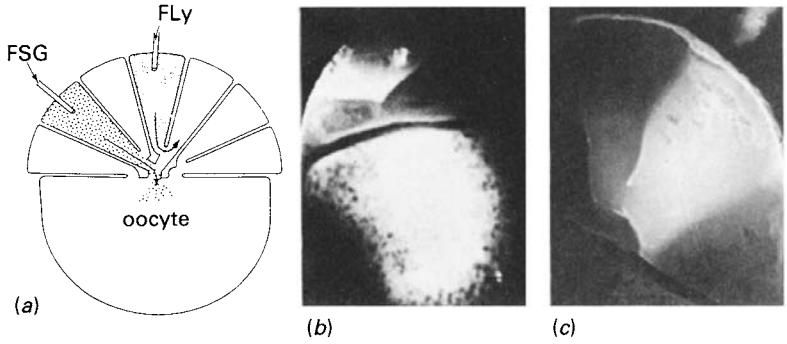

Fig. 1.3. The fate of fluoresceinated compounds injected into the nurse cells of the silkmoth *Hyalophora*. Acidic proteins like serum globulin, FSG (*a*) and methylcarboxylated lysozyme (*b*) are transferred to the oocyte, but lysozyme, FLy (basic), is not (*a, c*). Follicle diameters are 500 μm. ((*a*) from Woodruff & Telfer, 1980; (*b*) from Telfer *et al.*, 1981; (*c*) courtesy of Dr R. I. Woodruff.)

ported along quite long trophic tubes in telotrophic ovaries, and in many cases these contain a well-developed system of microtubules (Macgregor & Stebbings, 1970) although their role is not yet clear. In *Drosophila*, contractions by a microfilament system in the nurse cells seem likely to drive their contents into the oocyte (Gutzeit, 1986*b*). In several other species, a factor of importance for transport, and potentially for spatial determination, is the electrical polarity within the ovariole. Nurse cells are electronegative to oocytes, and proteins appear to be electrophoresed across the intercellular bridges: acidic proteins (negatively charged) moving from nurse cells to oocyte, and basic ones (positively charged) from oocyte to nurse cells (Woodruff & Telfer, 1973, 1980; Telfer, Woodruff & Huebner, 1981: see Fig. 1.3). Most soluble proteins and organelles are negatively charged and so should be driven into the oocyte, but positively charged proteins such as histones would require an acidic carrier if they are transported. Such an 'electrophoretic' system is highly unlikely to operate in *Drosophila* (Bohrmann *et al.*, 1986*a*,*b*), and it may well be that the problem of transport from the nurse cells has been solved in many different ways by different species (Gutzeit, 1986*c*).

There are instances where follicle cells also have open bridges with oocytes and appear to pass materials to them (lizards: see Andreuccetti, Taddei & Filosa, 1978), but usually they have no direct cytoplasmic contact with the oocyte. However, the membranes between the two cell types often show extensive interdigitation and specialised junctions and at least small molecules are able to pass between them (Browne, Wiley & Dumont, 1979). The follicle cells of mammals appear to promote the growth (Eppig, 1977) and block the maturation (Dekel & Beers, 1978; Larsen, Wert & Brunner, 1987) of oocytes in this way. Often the major

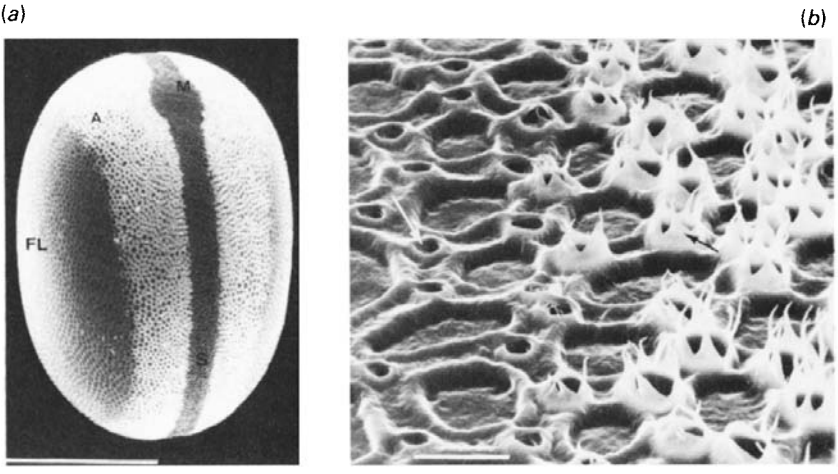

Fig. 1.4. Scanning electron micrographs of the outer surface of a silkmoth (*Antheraea*) eggshell after the removal of the follicular cells. (*a*) at low magnification four surface types are seen: flat (FL), aeropyle crown (A) stripe (S) and micropyle (M). Scale bar = 1 mm. (*b*), detail of the border between the FL and A regions in a tilted view. Scale bar = 10 μm. (From Mazur, Regier & Kafatos, 1982.)

products of follicle cell syntheses are components of the extracellular coats around oocytes (e.g. Paul *et al.*, 1972), and such coats may show spatial pattern. The silkmoth chorion, for instance, shows a characteristic pattern of surface sculpturing (Fig. 1.4) which is the result of a complex programme of follicle cell syntheses controlled both in time and space (Mazur, Regier & Kafatos, 1980; Regier, Mazur & Kafatos, 1980; Bock, Campo & Goldsmith, 1986). The elongated shape of most insect eggs also seems to be imposed by the follicular epithelium using a system of circumferentially oriented microtubules attached to desmosomes to resist circumferential expansion (Tucker & Meats, 1976).

Visible organisation and its origins

In the well-known case of the *Xenopus* oocyte, illustrated in Figure 1.1, spatial organisation first becomes obvious at stage II when cortical granules and pigment granules accumulate peripherally, producing an inside–outside or concentric organisation. At stage IV, polar organisation is also obvious as a gradient in the amount of yolk and the sizes of the yolk granules, and with the germinal vesicle and pigment granules restricted to the less yolky hemisphere (Dumont, 1972). These same two kinds of organisation are seen in many other types of oocyte; while in others only concentric patterns are ever obvious.

Spatial organisation of these kinds is almost always made visible by the graded distribution of protein yolk. Where yolk concentration increases from one pole to the other, the oocyte is said to be telolecithal. Yolk may be either denser (protein yolk) or less dense (lipid yolk) than other ooplasmic constituents, so a yolk gradient affects how released oocytes and eggs float. This, in turn, has resulted in further spatial differentiation of some oocytes – most obviously in amphibian ones which have pigmented upper halves and unpigmented lower ones so that the egg is cryptically coloured whether viewed from above or below. The origin of the yolk gradient here is apparently directed transport of platelets out of the upper half following uniform yolk uptake over the whole oocyte surface (Danilchik & Gerhart, 1987). In many groups, the germinal vesicle moves to the less yolky pole, and, when meiosis resumes, the polar bodies are given off here. The first cleavages also normally begin at this point, which early embryologists saw as the most active part. They also recognised the nutritional significance of the opposite yolky pole in many cases, and so described the two poles respectively as animal and vegetal. It is easy to understand the choice of such terms if we consider cases such as the chick where the whole embryo arises at the animal pole and grows by using the more vegetal yolk. Usually, the polar differences are far less dramatic than this, but most of our knowledge of spatial differentiation in oocytes still involves the animal–vegetal axis.

There are, however, some animal groups where the animal–vegetal axis is not obvious in oocytes. These include mammalian oocytes with little yolk and some other small oocytes with a central mass of yolk (centrolecithal oocytes). Such oocytes include those of ctenophores, ascidians and *Chaetopterus*. In such cases all other organelles are enriched in an outer ectoplasm. The best-known centrolecithal eggs are the characteristically elongated insect eggs where a central yolky area is surrounded by a relatively yolk-free periplasm. Here the polar bodies are given off close to one end of the egg, known only as the anterior pole, and the opposite end is called the posterior pole. The fact that oocytes and eggs exist which are not telolecithal shows that a yolk gradient cannot be an indispensable factor for the polarity of developmental potential which will be found to exist in most embryos. Polar organisation is detectable in the plasma membrane (Moody, 1985) and cortex (Schroeder, 1985) of the full-sized starfish oocyte, where it may only arise as the germinal vesicle migrates to the future animal pole. Insect oocytes provide the clearest examples of bilateral organisation, seen for instance in the detailed shape of the oocyte and in the exact position of its germinal vesicle (see Gill, 1964). In most other oocytes no dorso-ventral axis is recognisable, though the dorsal side of the unfertilised cephalopod egg is distinguishable by its shape (Watasé, 1891).

The distribution of yolk of course affects the distribution of all other