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Cytoplasmic domains in eggs

Christian Sardet, Alex McDougall and Evelyn Houlston

One way of organizing the body plan of a developing embryo is to establish domains in the egg with distinct compositions in defined spatial relationships to one another: when the egg divides up, these domains segregate preferentially to certain regions of the embryo and influence their development. In this review we discuss the nature, formation and reorganization of distinguishable domains in various eggs.

Different regions of the eggs of many animals are characterized by concentrations of particular organelles, such as pigment granules, mitochondria and ER, or exclusion of yolk platelets. These visible egg domains have been studied principally in insects (*Drosophila*), amphibians (*Xenopus*), ascidians (*Styela* and *Phallusia*), sea urchins and nematodes (*Caenorhabditis*), where their fate can be followed and/or their role in development tested by transplantation and ablation. Typically, egg domains are established in oogenesis in relation to the developing polarized oocyte structure, and are reorganized during oocyte maturation and following fertilization. This article concentrates on a comparison of cytoplasmic domains in different species during these early stages of development. The localization of mRNAs and proteins to particular domains and the role of cytoskeleton components in their organization have been discussed in recent reviews¹⁻³.

Establishment of domains in oocytes

The extent to which organizational asymmetry is established during oogenesis varies between animals (see Refs 4 and 5 for general discussions). In *Drosophila*, cytoplasmic and extracellular components

become localized along two axes in the oocyte, corresponding to the future anteroposterior and dorsoventral axes of the embryo⁶, and there is little shuffling of cytoplasmic domains following fertilization. By contrast, in amphibian and ascidian oocytes (Fig. 1 Ia and IIa), distinct domains are established about a single 'animal-vegetal' axis, in a radial arrangement^{7,8}, and sperm entry triggers important reorganizations. Other oocytes apparently have no domains relating to future embryo organization. In the mouse, for instance, cell interactions and cytoplasmic movements starting at the eight-cell stage are entirely responsible for patterning the embryo.

In *Drosophila*, the polarized organization of the oocyte arises during oogenesis as a result of its asymmetric spatial relationship with the cells surrounding it. There is only one obvious specialized cytoplasmic domain in the oocyte, a yolk-free region at the posterior end known as pole plasm^{9,10}. Other, 'invisible' localizations of specific proteins and mRNAs at the anterior and posterior poles are responsible for initiating a chain of events that determine the different fates of cells along the anteroposterior axis of the embryo^{5,6}. Pole plasm contains polar granules (ribonucleoprotein-rich bodies) associated with an accumulation of mitochondria, and is later inherited by certain 'pole cells', which found the germ line. A number of specific pole plasm components have been identified. A key component for the process of germ-plasm localization is *oskar* mRNA: its mislocalization causes other pole-plasm components to accumulate at the ectopic site and ultimately pole cells to form there¹¹. Distinct cytoplasmic domains with a similar appearance and function to pole plasm have been described in a wide range of organisms as 'germ plasm' and shown to contain germ-cell determinants^{9,10}. Furthermore, antibodies recognizing an RNA-binding protein in *Drosophila* polar granules detect germ-plasm components in *Xenopus*, suggesting that the molecular basis of germ-line determination may be conserved¹². Mitochondria, which are characteristically associated with germ plasm and have tightly associated mitochondrial large ribosomal RNA, may also contribute to the determinant properties of germ plasm¹³.

In *Xenopus* oocytes, germ plasm is associated with intermediate-filament proteins in the vegetal cortex⁷.

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It arises from a 'mitochondrial cloud' that moves from the centre of the oocyte to the vegetal cortex early in oogenesis. Patches of germ plasm spread from the cortex into the cytoplasm to form distinct islands during the hormonally triggered completion of meiosis. As in *Drosophila*, these are characterized by dense, RNA-containing 'germinal granules' and accumulations of mitochondria. The vegetal cortex also harbours specific mRNAs¹, whose protein products include growth factors and RNA-binding proteins. These are presumed to play roles in later development, although none has yet been demonstrated. Like the localized mRNAs in *Drosophila*¹⁴, they appear to use the polarized oocyte microtubule network to reach their final destination, where they may attach to cortical microfilaments or intermediate filaments. Differences in the timing of arrival and attachment of the different localized vegetal components suggest that several translocation and anchorage mechanisms operate. Another clearly recognizable domain in the *Xenopus* oocyte is established in the animal hemisphere during meiotic maturation¹⁵ (Fig. 1 Ia). This 'yolk-free domain' is rich in RNA and contains filamentous and vesicular elements, inheriting some of the nuclear contents as well as distinct material from around the base of the nucleus.

In ascidians, the contents of the oocyte nucleus also contribute to the formation of a large domain of clear animal cytoplasm. This 'ectoplasm' contains endoplasmic reticulum (ER), ribosomes, glycogen granules and fine filaments. Ascidian oocytes contain other visible domains, including 'myoplasm', present in a subcortical layer interrupted at the animal pole⁸ (Fig. 1 IIa). The myoplasm consists of a dense accumulation of mitochondria, an intermediate-filament network and, in some species, pigmented vesicles. It appears to assemble during oogenesis from an accumulation of pigmented vesicles and mitochondria very reminiscent of the amphibian mitochondrial cloud, and from an intermediate-filament network that develops beneath the microfilament-rich cortex¹⁶. Myoplasm is thought to contain determinants for the formation of muscle in the tadpole tail^{16,17}. Myoplasm is extensively modified or entirely absent in various species that do not develop into tadpole larvae with functional muscle cells¹⁶.

Immediate responses to egg activation

The immediate reorganizations of the egg cortex and cytoplasm that occur in response to egg activation (by fertilization, egg laying or parthenogenesis) appear mainly to be direct or indirect consequences of a transient increase in egg cytoplasmic Ca²⁺. At fertilization, this occurs as a wave of Ca²⁺ release from internal stores (mainly ER) into the cytoplasm, propagating from the site of sperm-egg fusion¹⁸. The Ca²⁺ transient triggers a wave of cortical reorganization, including the exocytosis of submembranous vesicles

called cortical granules (Fig. 1 Ia). Fragmentation of the ER network also occurs at the peak of Ca²⁺ release¹⁹.

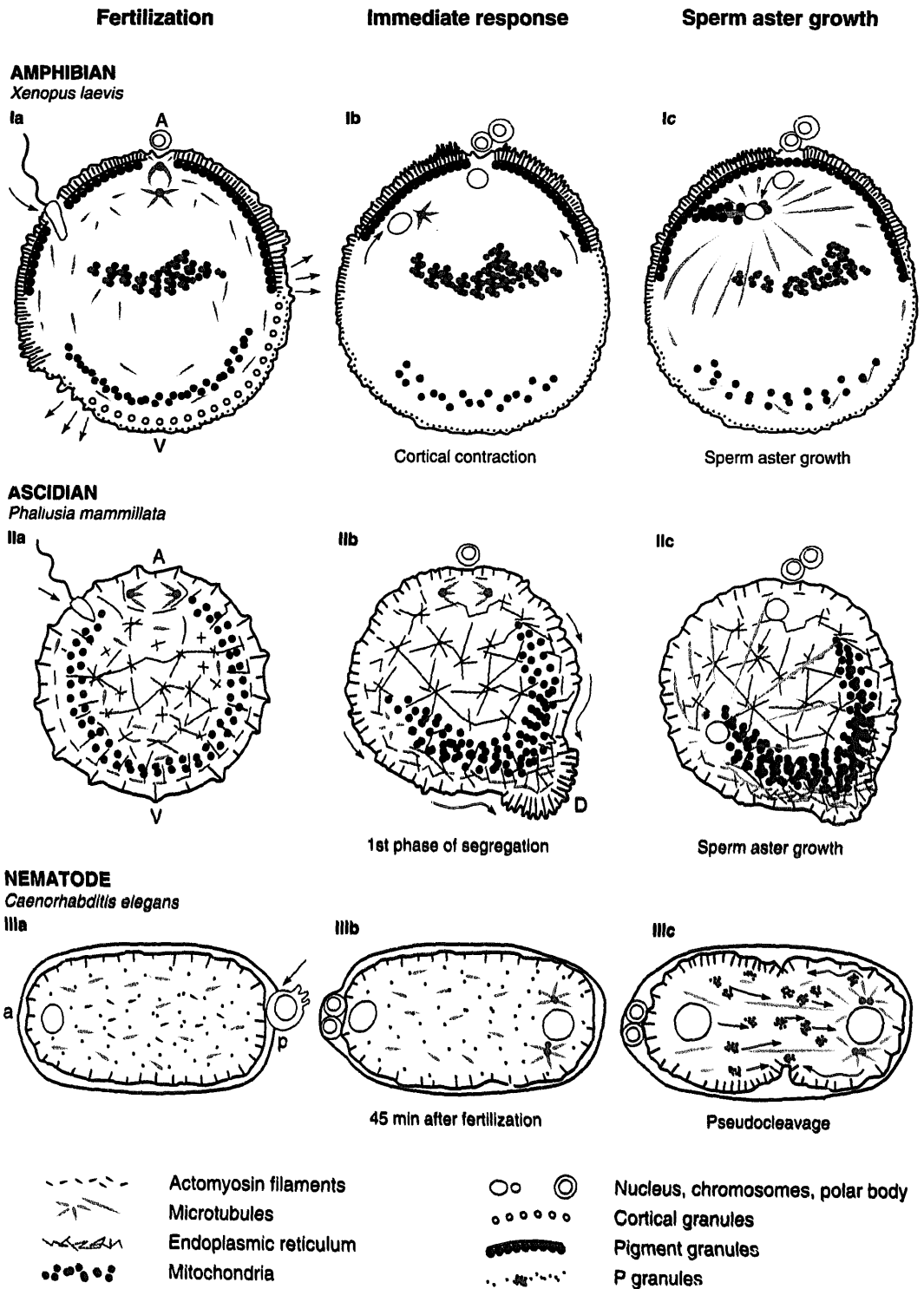
In sea urchin, as in other eggs, the Ca²⁺ transient is followed by a wave of actin polymerization and microfilament bundling. Microvilli become erect and there is a transient stiffening of the egg surface in response to the alkalinization of the cytoplasm, a consequence of the activation of Na⁺-H⁺ exchange by the Ca²⁺ transient^{20,21}. There is no obvious link between these cortical changes and later patterning of the embryo, but in some sea urchin species vesicles implicated in mRNA binding move into the cortex just after fertilization and are later preferentially released from the cortex of vegetal cells²².

In *Xenopus* eggs, cortical granule exocytosis and elongation of microvilli spread as a wave from wherever the sperm enters in the animal half (Fig. 1 Ia). The associated actin-based cortical contraction of the thickened pigmented cortex in the animal hemisphere (Fig. 1 Ib) appears to be centred around the animal pole, so does not break the radial symmetry of the egg. It brings the male pronucleus closer to the female pronucleus at the animal pole²³.

In *Phallusia*, the cortical contraction initiated by the Ca²⁺ wave is asymmetric, starting first on the side of the egg where the sperm entered²⁴ (Fig. 1 IIa and b). This contraction, which constitutes the 'first phase of ooplasmic segregation', involves a basket of cortical actomyosin microfilaments, open at the animal pole²⁵. It brings the sperm pronucleus, surface proteins and many cortical components towards a 'contraction pole' located to one side of the vegetal pole, on the opposite side to sperm entry²⁴. This asymmetric contraction results in the formation of a new domain, rich in microvilli and ER²⁶ (Fig. 1 IIb). The subcortical myoplasm domain is also pulled into the contraction pole region. UV irradiation or removal of small amounts of cytoplasm from around the vegetal pole of the *Styela* egg at this time can inhibit gastrulation, leading to the proposal that nucleic-acid-based axial determinants become concentrated transiently in this region²⁷.

Reorganizations during sperm aster growth and migration

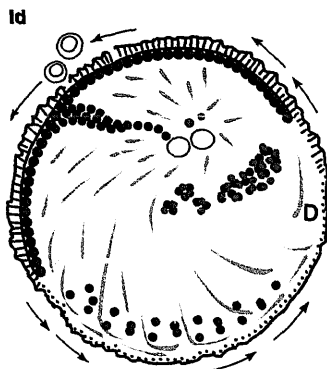
Sperm asters are giant asters of microtubules nucleated by the sperm-derived centrosome. They have been seen in most eggs examined, with the exception of mouse eggs²⁸. The microtubules of the sperm aster provide tracks for the female pronucleus to move towards the male pronucleus. Both pronuclei are taken by the growing aster to the centre of the egg (Fig. 1 Ic and d; IIc and d), a movement explained most simply as 'pushing' of growing microtubule ends against the cortex. Certain cytoplasmic organelles and parts of the ER network accumulate with the pronuclei in the center of the aster, while other



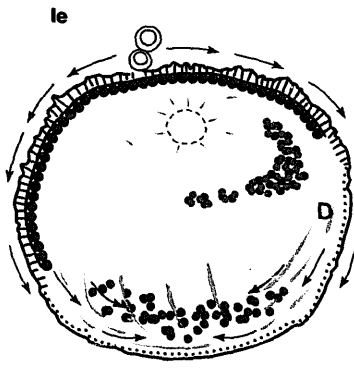
and migration

Mitosis

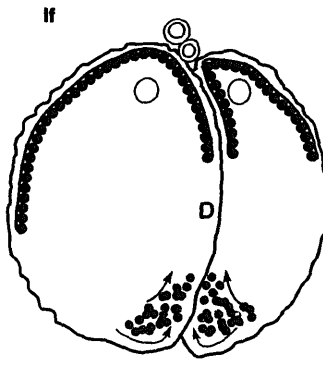
Cleavage



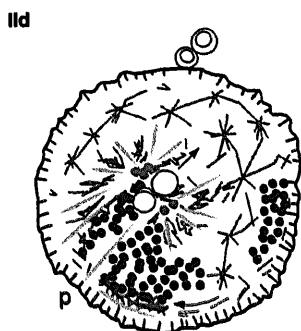
Cortical rotation



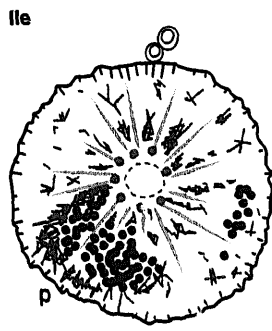
Surface contraction waves



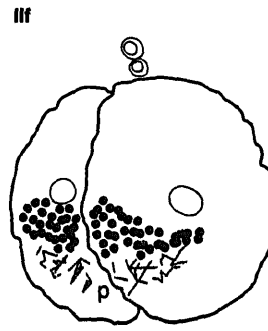
Simplified
3/4 dorsal view



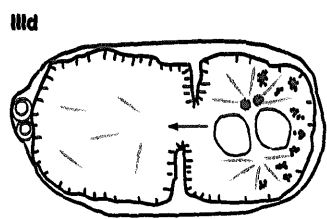
2nd phase of segregation



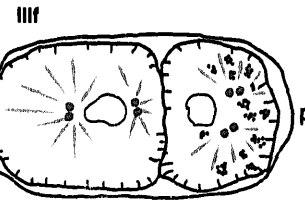
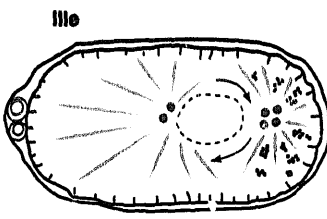
Rotation of nucleus







Simplified
3/4 posterior view



P-granule segregation



Unequal cleavage

-  Germ plasm
-  Microvilli
-  Vegetal yolk mass
-  Yolk-free domain

- A: animal pole
- V: vegetal pole
- D: dorsal
- a: anterior
- p: posterior

E 1

ic domains during early
s, *Phallusia mammillata* and
C. elegans.

organelles such as yolk granules are excluded. This formation of cytoplasmic domains in relation to the aster presumably results from plus- and minus-directed microtubule-based motor activity, both of which are favoured during interphase in isolated *Xenopus* egg cytoplasm²⁹.

In ascidians, reorganizations associated with the sperm aster elaborate the initial asymmetry created by the cortical contraction. The sperm aster grows in the vegetal hemisphere on the side of sperm entry, where the sperm nucleus and associated centrosome have been carried (Fig. 1 IIc). The centrosome flattens and moves to a sub-equatorial region, dragging the bulk of the ER-rich domain with it^{26,30}. The myoplasm is also dragged into the migrating aster, 'tearing' at the vegetal pole, to form a crescent of myoplasm marking the future posterior pole of the embryo (Fig. 1 II d and e).

The sperm aster of *Xenopus* plays an indirect and dispensable role in specifying the dorsoventral axis of the embryo. Microtubules extending from the aster join with an extensive array of aligned microtubules forming beneath the vegetal cortex³¹. This vegetal microtubule array appears normally to be oriented by the astral microtubules, but can form in the absence of a sperm aster and can be oriented independently of it by experimental means^{32,33}. Normal development can proceed if the sperm aster is eliminated³⁴. The aligned vegetal microtubules provide tracks for the translocation of the vegetal cortex relative to the underlying cytoplasm³⁵. During 'cortical rotation', the cortex appears to be transported to the plus end of the vegetal microtubules by a kinesin-related motor³⁶. The bulk movement of the vegetal cytoplasm relative to the cortex provokes complex swirling of the different domains in the more fluid animal cytoplasm³⁷ (Fig. 1 Id). Such relocation of egg components is ultimately responsible for giving dorsal characters (for instance the ability to induce the formation of dorsal mesoderm) to the cells that inherit the equatorial cytoplasm opposite the sperm entry point. Independently of the cortical-rotation-induced movements, segregation of yolk granules, cytoplasm and the yolk-free domain in the animal hemisphere occurs on sperm aster microtubules³⁸⁻⁴⁰. These movements do not appear to influence the establishment of the dorsal region.

In *Caenorhabditis*, the sperm aster also cues cytoplasmic localizations by indirect means⁴¹. A double sperm aster forms at the posterior end of the egg after the sperm has entered there and its centrosome has duplicated (Fig. 1 IIIa and b). Germ-plasm-like 'P granules' aggregate and segregate to the posterior pole during a period of cortical movements in the anterior half of the egg and the formation of a central pseudocleavage furrow (Fig. 1 IIIc). It has been proposed that the microtubules of the sperm aster promote a relaxation of cortical tension at the posterior pole⁴². The ensuing movement of the contractile

cortical elements towards the anterior pole would be balanced by the flow of central cytoplasm carrying P granules towards the posterior pole, where they are destined to segregate. This model explains the cytochalasin sensitivity of P granule movement at the time of pseudocleavage furrow formation.

Reorganization during mitosis and cleavage

The asters of the mitotic spindles can modify the organization of the egg cytoplasm by segregating certain cellular organelles such as ER and yolk vesicles. Directed cytoplasmic movements around the time of mitosis also take place as a result of 'precleavage waves', or 'surface contraction waves' (SCWs). These were described first in amphibian eggs, where they originate at the animal pole⁴³. In *Xenopus*, SCWs cause the islands of germ plasm situated below the vegetal cortex to accumulate around the vegetal pole, by a microtubule-dependent mechanism⁴⁴ (Fig. 1 Ie and f). Similar waves of reorganization, including microtubule-dependent cytoplasmic remodelling, occur before cleavage in the ctenophore *Beroe*⁴⁵. These waves appear to reflect the localized activation of mitotic factors around the zygote nucleus. The pre-cleavage waves in *Beroe* occur at the time the single embryonic axis is specified, and run in the direction of this axis; however, no causal link has yet been established.

Exaggerated actin-based surface contractions and deformations at the time of mitosis in many mollusc and annelid eggs result in the formation of 'polar lobes' of various sizes near the vegetal pole⁴⁶. At each of the first few cleavages, the polar lobe and its contents join only one cell, allowing their constituents (distinct vesicles, germ-plasm-like material, RNA-rich cytoplasm) to be segregated, and thus direct the fate of different regions of the embryo. Polar lobe formation involves a microfilament-dependent constriction like a cleavage furrow, but is a distinct process⁴⁷.

Results from studies of eggs and early embryos from a number of invertebrates with stereotypic cleavage patterns (including ctenophores, annelids and molluscs) emphasize the importance of cleavage itself in distributing cytoplasm with distinct developmental potentials in the embryo⁴⁸. In many of these cases, peripheral cytoplasm can be observed to relocate with the progressing furrow. Unfortunately it is difficult to attribute the redistribution of developmental potential to furrow formation, rather than to the spindle structure, pre-cleavage waves or other tightly linked events. Similar ingression of peripheral cytoplasm during cleavage in *Xenopus*³⁹ (Fig. 1 Ie and f) is implicated in moving germ plasm deeper into vegetal blastomeres⁴⁴. The extensive mixing of cytoplasm from different regions of the egg resulting from such ingression may well have other consequences in bringing together egg components originally localized in different regions.

Common mechanisms in domain establishment?

Can any general conclusions be drawn from this examination of different egg cytoplasmic domains? Accumulations of mitochondria are found in several types of domain (e.g. amphibian germ plasm and ascidian myoplasm), and ER accumulations have been described frequently in asters. We can speculate that the accumulation of organelles such as ER and mitochondria that affect cytoplasmic Ca²⁺ concentration and pH could help establish distinct 'physiological domains' inherited by specific blastomeres with different fates. Certain associations of organelles are probably favoured in eggs as the result of the activity of motor proteins that move different organelles over polarized frameworks of cytoskeletal filaments. However, there are many differences in the details of organelle-cytoskeleton associations between different eggs. Mitochondrial domains can form in microtubule asters, be linked to the actin cortex (e.g. in *Phallusia*), or be associated with intermediate filaments. Furthermore the composition and structure of the most widely found domain, germ plasm, varies from animal to animal and between different stages of development⁹. In *Drosophila*, mitochondria are preferentially active in the vicinity of germ plasm, even when they are not concentrated there⁵³. Additional markers for specific organelle subpopulations (differences within the ER for instance), as well as localized mRNAs and proteins, will be required before we can decide whether definable 'domain phenotypes' exist.

Once formed, egg domains tend to move as blocks rather than organelle by organelle. Displacement mechanisms thus rely on bulk flow or movements of large structures rather than transport of individual components. Understanding the translocations will require both characterization of organelle-cytoskeleton interactions, and understanding of cellular fluid mechanics. The creation of new domains or the juxtaposition of pre-existing domains after translocation could allow developmentally important components from different parts of the egg to interact. In *Xenopus*, cytoplasmic movements associated with cortical rotation and cleavage may bring the products of vegetally localized growth factor mRNAs, originally attached to the oocyte cortex, to an equatorial region to be processed (perhaps proteolytically cleaved or secreted)^{50,51} by components originally localized in the animal yolk-free domain. Such ways of creating regional differences in embryos could be considered as 'intracellular induction'.

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The TGF- β family and its composite receptors

Joan Massagué, Liliana Attisano and Jeffrey L. Wrana

In their search for regulators of animal growth and development, biologists have often come upon members of the transforming growth factor β (TGF- β) family and have realized that these are among the most versatile carriers of growth and differentiation signals. New evidence suggests that these factors signal through receptors with remarkable structures. Each receptor is a complex of two distantly related transmembrane serine/threonine kinases that are both essential for signalling. TGF- β and related factors have at their disposal a repertoire of such receptors, a feature that could account for their multifunctional nature.

TGF- β and related polypeptides regulate cell growth, differentiation, motility, organization and death. Some of them participate in setting up the basic body plan during early embryogenesis in mammals, frogs and flies, whereas others control the formation of cartilage, bone and sexual organs, suppress epithelial cell growth, foster wound repair, or regulate important immune and endocrine functions. The TGF- β family is therefore viewed as one of the most influential groups of growth and differentiation factors. Elucidating the mode of action of this family should unveil regulatory mechanisms of considerable interest. In the past two years, this area has seen major advances, with the identification of various

TGF- β receptors and downstream targets of the signals that they impart. This article focuses on the field's progress and directions.

Structure of the factors

Over two dozen TGF- β -related factors have been identified. The biologically active forms are disulphide-linked dimers containing subunits of 110-140 amino acids. The subunits are synthesized as the C-terminal portion of larger precursors^{1,2} (Fig. 1a). After dimerization, the precursor is cleaved at a cluster of basic residues by a furin peptidase in the secretory pathway, which releases the bioactive domain. This domain may remain noncovalently associated with the propeptide, yielding a latent complex that is unable to bind receptor but can be activated later in the extracellular medium.

All family members show sequence similarity to the prototype, TGF- β 1, particularly in the active domain, where the most conserved feature is the spacing of seven cysteines. The crystal structure of TGF- β 2 reveals that six of these cysteines form a rigid central structure known as the cystine knot³ (Fig. 1b). This singular motif is an eight amino acid ring closed by two cystine disulphide bonds and spanned by a third one. Four segments of twisted antiparallel β -sheets extend from the knot like two slightly curved fingers, and an α -helix extends opposite to the fingers like the heel of a hand^{4,5}. The remaining cysteine forms a disulphide bond with the corresponding cysteine in the other monomer.

The two monomers are oriented flat antiparallel to each other, and the inner surface of the fingers in one monomer forms hydrophobic contacts with the heel of the other^{4,5}. These contacts might be sufficient to dimerize GDF-3 or GDF-9, two members of the TGF- β family that lack the seventh cysteine⁶. Certain unconserved residues at the fingertips and back of the hand could constitute receptor contact sites^{4,5}. TGF- β s and activins, but not other family members, contain an extended N-terminal region with two cysteines. In TGF- β 2, this region forms a short α -helix

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