Germ Plasm Revisited and Illuminated

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INFORMATION IS NOW ACCUMULATING TO HELP EXPLAIN THE century-old mystery of the mechanism for localizing developmental information in the oocyte. The segregation of ooplasmic substances can determine specific cell types. The transplantation of posterior polar plasm from the *Drosophila* egg, which induced ectopic germ cells, demonstrated that this ooplasm was an autonomous determinant for primordial germ cell determination (*1, 2*) and that this information was already preformed during oogenesis (*3*). Detailed ultrastructural studies indicated that the polar granule was a distinctive organelle of the germ plasm (*4*), found continuously throughout the life cycle of the germ line (*5*). During oogenesis polar granules accumulated in the posterior polar plasm and stained positively for RNA (*6*). After fertilization the granules acquired polysomes at their surface and dispersed in the posterior polar plasm. After pole cell formation, polar granules reaggregated into a few large granules, which no longer stained for RNA. Then, the granules again fragmented and associated with the outer nuclear envelope as "nuage" (*5, 6*). Polar granules have been purified from pole cells and shown to have a 95-kilodalton basic protein (*7*). Mitochondrial ribosomal RNA inexplicably mimics some of the inductive properties of polar plasm (*8*).

Despite the ability of the posterior germ plasm to function autonomously, maternal effect mutations that affect only the formation of germ cells are rare (*9, 10*). However, recent genetic screens have identified a class of maternal genes (*10*) that is required for abdominal region development (termed the posterior class) and for pole cell production. Two genes of the posterior class, *nanos* (*nos*) (*11*) and *pumilio* (*pum*) (*12*), are essential for abdominal development only, whereas the remaining eight are also required for active posterior germ plasm production. The polar granule may be the common element for the development of the abdominal region and the germ cells. One of these eight genes, *vasa* (*vas*), has been shown by immunoelectron microscopy to be a component both of the granule and nuage (*13*). Many of the others appear to be essential for assembling polar granules, an organelle required for localizing *nos* RNA, the posterior determinant (*14*). Understanding the interaction of each gene with the other genes and the germ plasm makes proposing a pathway for the assembly of the polar plasm possible.

Oogenesis in *Drosophila* involves the generation of a set of 16 interconnected sister cells in the germarium at the anterior tip of the ovary. One of these 16 cells is specified as the oocyte; the rest develop into nurse cells. These latter cells become polyploid and synthesize most ooplasmic components (*15*). Polar granules appear during vitellogenesis, although nuage-like structures related to polar granules associate with nurse cell nuclei at earlier stages (*4*). Both *oskar* (*osk*) (*16, 17*) and *tudor* (*tud*) (*18*) RNAs accumulate in the oocyte even before the 16-cell cyst leaves the germarium. At the start of vitellogenesis, *tud* RNA is found only in the oocyte; later, *tud* RNA disappears from the oocyte and is found in the nurse cells. In contrast to *tud* RNA, *osk* RNA remains primarily in the oocyte and during vitellogenesis assumes a transitory localization as an anterior ring around the periphery of the oocyte before becoming exclusively localized to the polar plasm. A pattern of RNA localization, similar to *tud* RNA, is displayed by *staufen* (*stau*) (*19*) RNA because it initially is localized to the oocyte but during vitellogenesis becomes restricted to the nurse cells. Localization of the Staufen protein mimics that of the *osk* RNA. It forms an anterior ring in the oocyte during early vitellogenic stages and later localizes to the polar granules. Oskar protein appears to be required to maintain the localization of both *osk* RNA and Staufen protein to the polar plasm because three different *osk* alleles, each having a stop codon that presumably results in a truncated protein (*17*), fail to sustain the accumulation of either *osk* RNA or Staufen protein. As progressively stronger *tud* alleles have correspondingly less polar granule material (*20*), it is possible that the Tudor protein also contributes directly to the polar granule.

Two genes, *capu* and *spir* (*21*), which have not yet been cloned, appear to act early in assembling the polar plasm. Both are required for the localization of Staufen at the posterior tip (*19*); moreover, *capu, spir*, and *stau* are required for *osk* RNA localization (*17*). Because *capu* and *spir* mutations affect posterior, anterior, and dorsal-ventral pattern, these loci are probably required for the general organization of the egg. A recently isolated gene, *mago nashi* (*mago*) (*22*), shows the combined posterior and grandchildless phenotype (including a reduction in the amount of polar granules) and the spatial coordinate phenotype characteristic of bicaudal embryos. Temperature shift experiments indicate that the embryonic phenotype is dependent on *mago* function during vitellogenesis, the period when polar granules are assembled.

Early models of polar granule function postulated that the organelle was a repository for messenger RNAs (*23*), and this has been validated for both *osk* and *nos* RNAs (Fig. 1). There are probably other RNAs specifically needed for germ cell formation that are also stored in the granule. Eight of the ten posterior genes are required for producing the granule, and the effect of double mutations suggests a sequence of action. The general structure of the egg, including the posterior polar plasm, requires *spir* and *capu*; *stau* is required for initiation of *osk* messenger RNA localization, but Oskar protein itself is needed to maintain both Staufen protein and *osk* messenger RNA in the polar plasm. Because mutations in *valois* (*vls*) affect neither *osk* RNA nor Staufen and Vasa protein localization, *vls* probably lies downstream of these functions. The role of *tud* is not as clear because none of the extant alleles is a null. Because the strength of the *tud* allele correlates with the amount of polar granules, *tud* is probably involved in polar granule assembly (*17*). Except for *vas*, whose putative amino acid sequence indicates that it belongs to the Asp-Glu-Ala-Asp (D-E-A-D) family of RNA-depen-

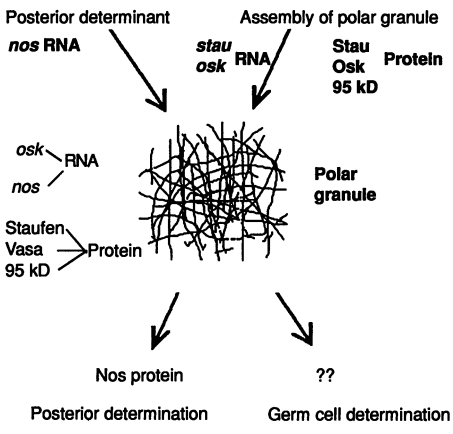

Fig. 1. Polar granule functions.

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dent adenosine triphosphatases (*24*), none of the gene products has known homologies. Biochemical evidence indicates that polar granules, isolated from pole cells, have a major component of approximately 95 kilodaltons (7), but none of the cloned posterior group genes codes for a protein of this size.

In spite of the association of the posterior polar plasm with germ cell determination, none of the posterior group genes appears to be specifically responsible for pole cell formation. The presence of polar granules remains the common component essential for a functional germ plasm and for the posterior localization of *nos* RNA. Thus, there are probably additional gene products that are key to germ cell formation but may have little effect on posterior development. Saturation screens for true grandchildless mutations remain to be conducted. Organelles comparable to polar granules are also found in the amphibian germ plasm (*25*) and may be present in other organisms (*26*). These organelles may have multiple functions wherever they are found.

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β Ribbon: A New DNA Recognition Motif

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THE TWO MOST STRIKING FEATURES OF DNA ARE ITS BEAUTY and symmetry. The beauty is displayed by the curvature of the right-handed double helix and the symmetry arises from the two twofold rotation axes per base pair, one in the plane of each base pair and the other between every two adjacent base pairs. Both twofold axes are perpendicular to the helix (Fig. 1A). Since these two features are so striking, it was widely recognized that they would be used by proteins that recognize DNA.

What types of symmetries do protein structural elements such as α helices and β sheets have? An α helix has neither a twofold axis nor a helical curvature comparable to double-helical DNA. However, an antiparallel β ribbon (two-stranded β sheet) contains two types of twofold axes (Fig. 1B) that have separations comparable to those of the twofold axes of double-helical DNA. The twist curvature is also comparable to that of DNA. This striking similarity prompted molecular modeling of the recognition between a β ribbon and double-helical RNA (*1*) and DNA (*2*), in which the curvature and symmetry axes of the β ribbon and double-helical DNA were matched (Fig. 1C).

When crystallographic evidence for such a model was sought by soaking short peptides such as protamines into transfer RNA crystals, it came as a surprise that the α helix rather than the β ribbon was bound in the minor groove of a double-helical region of transfer RNA (*3*). The real surprise came when higher resolution crystal structures of DNA complexes of several DNA-recognizing proteins were determined [reviewed in (*4*)]. In all of these structures, DNA was recognized by a helix-turn-helix motif in which neither the symmetry nor the helical curve of DNA was used by the recognizing protein. More recently, the structure of another DNA recognition motif, the zinc finger, revealed that the α helix again was the recognition element (*5*). It almost appears as if nature has taken the second best option. What advantage do these motifs have as a structural element for recognizing double-helical DNA? These motifs have neither helical twist nor symmetry elements similar to those of DNA. The examination of the helix-turn-helix motif in various different DNA complexes reveals that the first helix serves as a stage on which to present the second helix, which protrudes out of the protein to interact with the DNA grooves. In the zinc finger motif, a zinc ion holds the β ribbon and the α helix together to form the entire peptide into a compact package. In both cases, the recognition helix orients in such a way that the positively charged amino-terminal end of the helical dipole points toward the groove, which is surrounded by the negatively charged phosphates of DNA.

A recent crystallographic study of MetJ repressor complexed with DNA by Somers and Phillips (*6*) finally revealed the antiparallel β ribbon as a DNA recognition motif, as was predicted from nuclear magnetic resonance studies of Arc repressor by Breg, Kaptein, and their colleagues (7). The crystal structure shows that the motif recognizes the major groove of double-helical DNA, bringing the twofold symmetry axes of the ribbon coincident with those of double-helical DNA (the upper option in Fig. 1C is assumed by the repressor dimer, as shown in Fig. 1D).

The MetJ repressor is a dimer of identical 104-residue subunits, binds two corepressors, *S*-adenosyl-L-methionine, noncooperatively, and recognizes each eight–base pair "met box" in six known operators (each operator contains two to five "met boxes") that negatively regulate the expression of the enzymes of the methionine biosynthetic pathway in *Escherichia coli*. In the crystal structure, an amino-terminal β strand of ten residues (residues 20 to 29) of one monomer and its twofold symmetry-related counterpart of the other monomer form a β ribbon. This ribbon binds to the compressed major groove of an operator DNA so that the two types (types a and b in Fig. 1B) of twofold axes of the ribbon coincide with both types of twofold axes of the DNA (Fig. 1A). The amino acid side chains on the DNA-facing side of the ribbon recognize the edges of the base pairs on the major groove or phosphate. Specifically, a set of hydrogen bonds are formed between Thr^{25} and N7 of an adenine, Lys^{23} and N7 of a guanine, and Lys^{22} and a phosphate oxygen and another set of hydrogen bonds related by a twofold axis (Fig. 1E). If a β ribbon is bound to the minor groove, similar base recognition might be difficult because of the

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