Embryonic Transcription and the Control of Developmental Pathways

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> It appears that the initial steps up to the [sea urchin] blastula stage are independent of the quality of the nuclear substance, even though it is essential that the nuclear substance be of a kind capable of existing in the egg. The necessity for particular chromosomes becomes apparent first with the formation of the primary mesenchyme and from then on shows up in all processes as far as development can be observed.... With respect to those characters in which we are able to recognize individual variations, the nuclear substance and not the cytoplasmic cell substance imposes its specific character on the developing trait.
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> ... Earlier stages, for which according to our results, specific chromosomes are not necessary, demonstrate a purely maternal character.... I would like to ascribe to the cytoplasm of the sea urchin egg only the initial and simplest properties responsible for differentiation... it provides the most general basic form, the framework within which all specific details are filled in by the nucleus. THEODOR BOVERI 1902

EVEN before the molecular nature of the gene was understood, embryos were known to get the products they need from two different sources. In modern terms, the unfertilized egg contains large stores of maternal RNA and protein that are derived from transcription during oogenesis. Initially, these proteins provide the basic machinery for all cellular events. At some point these maternally supplied products are supplemented by transcription from the embryo's own genome (the "nuclear substance" in BOVERI's formulation). This occurs at different stages in different organisms, but significant transcription is usually detected by the blastula or blastoderm stage. Maternal gene products persist after this point, however, and most processes during embryogenesis involve both maternal and zygotic components.

As the quote from BOVERI's classic paper indicates, embryologists at the turn of the century were aware of both maternal and zygotic contributions to embryonic development. They even considered the possibility that maternal components might play different roles in development than zygotic components. In the eighty years that followed BOVERI's work, it became clear that certain zygotically active genes play major controlling roles. Landmark studies were POULSON's (1940) analysis of chromosomal deletions in the *Notch* region and LEWIS' (1978) characterization of the Bithorax complex.

In the following pages, I will address the generality of a hypothesis only partly implied by the BOVERI quote, namely that in organisms where zygotically required gene activities are rare, all such activities have been selected to play unique, controlling roles in development. In this admittedly extreme reformulation of BOVERI's observation, I will restrict consideration to those genes whose products *must* be supplied zygotically and to organisms that have minimized their transcriptional requirements during embryogenesis. I will first present reasons why such a division of labor makes teleological sense and then examine the data from Drosophila to determine the extent to which gene activity actually obeys these expectations. Then I will discuss the exceptions, *i.e.*, certain well-characterized cases where particular Drosophila genes are supplied zygotically but do not seem to play controlling roles. Lastly, the Drosophila data will be compared to that from other organisms.

The simple model: In organisms where embryonic development is rapid and occurs with no increase in size before hatching from the egg, it will be advantageous to maximize maternal contributions, because the duration of oogenesis is often much longer than embryogenesis and the ovary provides a more sophisticated and efficient synthetic machinery. Evolutionary selection for efficiency will maximize such maternal contributions. One major limitation to maternal supplies is the duration of embryonic development. Maternal supplies must be sufficient to last until a larva hatches and can obtain additional nutrients from the environment. In principle, maternal contributions might consist only of nutrient material ("yolk") that could be converted by the embryo to a wide variety of gene products. It may be more efficient, however, that the "nutrients" include RNA directly encoding the individual components of most biological processes, including the cellular machinery required for transcription, translation, energy utilization and basic cell structure.

On the other hand, all embryos have been shown to require certain RNAs supplied by transcription of their own genome. Early indications of this requirement came from the seminal work of BOVERI, as well as species hybridization experiments and actinomycin and α-amantin studies (reviewed in DAVIDSON 1986). If supplying gene products maternally is so advantageous, what can zygotic transcription do that just storing maternal transcribed gene products can't? One possibility is that zygotic transcription allows much more precise expression, putting particular gene products in one cell but not in its immediate neighbors. Such precision is probably not necessary for most gene products. If a gene product is needed in some cells but not others, it may be sufficient (although profligate) to supply it uniformly to

the whole egg. A patterned expression only becomes important when the *absence* of a particular gene product is as significant as its presence. This might be the case if the gene product is used as a developmental switch, such that its expression in one cell causes that cell to assume a fate different from its neighbors. While it is possible to localize maternal gene products to particular regions of the egg cytoplasm, local transcription may allow more precision, and may therefore be more useful as spatial patterns become complex.

This view has certain practical consequences. Over the course of evolution, the efficiencies of maternal contribution will reduce zygotic transcription to a minimum. That minimum will define a set of genes, each of which is limiting for a specific process, such that its presence or absence determines when and where in the embryo that process takes place. Not all controlling elements will be zygotic; a process could be initiated by a localized maternal RNA or ligands (ST JOHNSTON and NÜSSLEIN-VOLHARD 1992). However, if there is a zygotic component in a process, its expression is likely to play a controlling role in all events downstream to it. In this view, selection during evolution has solved the research agenda of a large fraction of developmental biologists: in a complex process involving many gene products, it has sorted out which one actually plays the controlling role. Therefore, to understand how a particular process is controlled in the embryo, good candidate genes would be those whose products need to be supplied zygotically. For organisms where genetic analyses are possible, identification of candidates is relatively straightforward; if the genes are removed by mutation, homozygous mutant embryos will develop abnormally.

Consistent with behavior as controlling elements, genes that must be supplied zygotically represent only a small fraction of the Drosophila genome: The mutagenesis screens that CHRISTIANE NÜSSLEIN-VOLHARD, GERD JURGENS and I began more than fifteen years ago in Heidelberg offered an initial opportunity to test whether the expectations outlined above apply to Drosophila (NÜSSLEIN-VOLHARD and WIESCHAUS 1980; JURGENS *et al.* 1984; NÜSSLEIN-VOLHARD *et al.* 1984; WIESCHAUS *et al.* 1984). Of the 18,000 lethal mutations induced in those screens, only about 4500 (25%) cause death during embryogenesis, and only 580 cause alterations in visible morphology sufficient to allow homozygous embryos to be distinguished from their heterozygous siblings. These 580 mutations were assigned to 139 complementation groups with an average hit frequency of 4.2. Identification of mutants in the Heidelberg screens was based on examination of the larval cuticle. Later screens using other aspect of general morphology (EBERL and HILLIKER 1988) or using molecular markers to follow the development of specific organ systems (SEEGAR *et al.* 1993; VAN VACTOR *et al.* 1993; HARBECKE and LENGYEL 1995) identified additional genes, although their number is not large. The major conclusion from all these studies is that only a small number of loci need be transcribed in the embryo itself to establish normal morphology. Even if the Heidelberg screens detected only half the zygotically important loci, the number of such genes would increase only to 300, fewer than 2% of the estimated 20,000 molecularly defined transcription units and only a small fraction of the transcripts and proteins found in the embryo. The remainder of these proteins and RNAs, and thus the majority of the components involved in any particular embryonic process, must be supplied maternally.

If early acting, zygotically transcribed genes are rare, it should be possible to delete large portions of the genome with little effect on early stages of development. We tested this possibility by examining the development of embryos homozygous for various cytologically defined deletions, and eventually used translocations and chromosomal rearrangements to generate embryos deleted for overlapping regions spanning the entire Drosophila genome (MERRILL *et al.* 1988; WIESCHAUS and SWEETON 1988). In general, such deficiency embryos show phenotypes that could be explained by point mutations previously located to the deleted regions. There are exceptions, notably a group of seven early acting genes required for cellularization at the onset of cycle 14. Ongoing work in my lab suggests the existence of certain regions with previously undescribed effects on gastrulation and early morphogenetic movements (E. WIESCHAUS, unpublished results). In general, however, such newly discovered loci have been rare, confirming the preponderance of maternal gene products and scarcity of gene products that must be supplied by zygotic transcription.

Transcripts that must be supplied to the embryo zygotically appear to play special roles in development: Most of the mutations identified in the Heidelberg screens produce discrete phenotypes, such that differentiation of most structures is normal and defects are limited to specific cell types or regions. Also, most phenotypes are locus-specific (*i.e.*, there is only one locus in the genome that can be mutated to produce that phenotype). Subsequent molecular analyses suggest that this specificity is often reflected in their expression patterns during embryogenesis. On the *X* chromosome, for example, where 14 of the 20 loci have been cloned, 11 show patterns of transcription corresponding to where the gene products are required in the embryo. Genes that show uniform phenotypes affecting all cells at a particular stage (the early acting cellularization genes *nullo*, *sryα* and *bottleneck*) show uniform transcript distribution, but are only expressed in a short period immediately preceding cellularization (JAMES and VINCENT 1986; ROSE and WIESCHAUS 1992; SCHEJTER and WIESCHAUS 1993).

Another feature of these genes that suggests specialized functions is that their transcription is generally not required during oogenesis. This behavior is different

from most genes in Drosophila and is best illustrated by examination of the *X* chromosome, where the wild-type activities associated with many random lethal loci have been studied using germ-line clones (PERRIMON *et al.* 1984, 1989). Most of these lethals (35/48 and 133/211 in two separate surveys) cause blocks during oogenesis or abnormalities in embryogenesis that cannot be corrected by zygotic transcription. In contrast, only three of the 19 Heidelberg loci that have been tested are required during oogenesis (JIMENEZ and COMPOS-ORTEGA 1982; ZUSMAN and WIESCHAUS 1985; WIESCHAUS and NOELL 1986; EBERL *et al.* 1992). For the remaining 16 loci, a single zygotic allele is sufficient to ensure normal development of the embryo, even when all maternal activity is removed by producing germline clones. In several cases (*mys, exd, fog*) alterations in the development of the resultant progeny suggests that the wild-type genes may normally be transcribed during oogenesis. Unlike the average vital gene, however, such transcription is not necessary for embryonic survival, as long as the embryo has at least one normal copy of the gene.

The precision of these expression patterns might simply reflect an economy of synthesis, the embryo only making gene products where it needs them. In a few cases this possibility has been excluded by expressing the gene ectopically. This was initially accomplished for the *ftz* segmentation gene using a heat-shock promoter (STRUHL 1985), but alternative systems such the Gal4/UAS system of BRAND and PERRIMON (1993) have been designed to produce more specific patterns of expression. If the presence of a particular gene product directs a cell into a fate different from that of its neighbors, then it should be possible to direct its neighbor (or any other cell) into the same fate by forcing expression of the same gene product. Although only a few genes have been tested, the results have confirmed the significance of most of the spatial expression patterns associated with genes affecting all three levels of segmentation (PARKHURST and ISH-HOROWICZ 1991; CAPOVILLA *et al.* 1992; MANOUKIAN and KRAUSE 1992; NOORDEMEER *et al.* 1992; TSAI and GERGEN 1994), as well as neuronal development (RHYU *et al.* 1994), cell proliferation (EDGAR and O'FARRELL 1990) and gastrulation (P. MORIZE, M. COSTA, S. PARKS and E. WIESCHAUS, unpublished results). One potential exception (*patched*) is discussed below.

Do all zygotically required genes play controlling roles? The observations presented above suggest that most transcriptionally required genes play specific regulatory roles in the Drosophila embryo. There are exceptions, of course, but many of the apparent exceptions can be integrated into the model with little modification. As mentioned above, some of these genes are also transcribed during oogenesis. In many such cases, the maternal products appear to be supplied in insufficient quantities, so that patterned zygotic transcription still provides the critical difference in establishing cell behavior (WIESCHAUS and NOELL 1986). Such maternal transcripts may bring all cells close enough to the threshold so that small differentials in zygotic transcription between neighboring cells can make an immediate difference.

At the other end of the spectrum are gene products required throughout embryonic development in such large quantities that maternal supplies are not sufficient and additional zygotic transcription is required by later stages in development. Such genes may represent the bulk of embryonic-lethal mutations that make it through the last major morphological events of Drosophila embryonic development (dorsal closure and head involution), but die before hatching with no obvious visible defects, a class that we found represented about 25% of all lethal genes. In some cases the requirement for additional zygotic transcription may set in earlier, so that mutations cause visible defects in late embryonic processes. The major cytoplasmic myosin gene (*zipper*) may provide an example (YOUNG *et al.* 1993). Although cytoplasmic myosin is required throughout development, mutations that block zygotic transcription cause abnormalities only during dorsal closure and head involution. Zygotic transcription of *zipper* does not appear to play a regulatory role; *zipper* transcripts are uniformly distributed in normal embryos, and the mutant defect can be rescued by ubiquitous *zipper* transcripts supplied from a heat-shock promoter (YOUNG *et al.* 1993). Presumably the early normal development of homozygous mutants reflects maternal myosin RNA and protein that begin to run out shortly before dorsal closure.

More puzzling, however, are cases where the absence of zygotic transcription produces an early phenotype (within the first two hours after the blastoderm stage), yet the relevant gene product is transcribed uniformly in the embryo. Examples include *arm* (RIGGLEMAN *et al.* 1990), *Notch* (JOHANSEN *et al.* 1989), *exd* (RAUSKOLB *et al.* 1993) *odd-paired* (BENEDYK *et al.* 1994) and *flb/DER* (LEV *et al.* 1985; ZAK *et al.* 1990). The uniform distribution of these transcripts makes it difficult to see how their transcription could play a controlling role in choosing between pathways. Some of these genes (*Notch, flb/DER*) encode cell surface receptors or (*armadillo*) other molecules required for the response of cells to local signals. Many of these signals are known; they are encoded by other zygotically active genes that are expressed in restricted patterns, such as *wingless* (BAKER 1987). It is natural to assume that it is the expression of these latter genes that controls cell fate. The early stage of development when signalling is required makes it hard to understand why their receptors could not be supplied maternally.

In some cases, a large supply of maternal receptor protein may be disadvantageous if that receptor can cross-react with or interfere with processes that involve similar components. The Drosophila EGF receptor (*flb/*

DER), for example, is required in the follicle cells but not in the oocyte during oogenesis (SCHÜPBACH 1987). It also utilizes many of the same downstream elements (*e.g.*, Ras, Raf) as certain maternally supplied receptor tyrosine kinases (torso) that pattern the embryo during cleavage; see DUFFY and PERRIMON (1994). To avoid cross-reactions, it may be simplest for the organism not to express *flb/DER* transcripts in maternal germ cells. This would explain why *flb/DER* is not expressed maternally and only expressed zygotically after the blastoderm stage. This notion could be tested by determining whether maternally supplied *flb*/DER product has deleterious effect on development or, alternatively, can rescue mutant embryos. In any case, it is more difficult to apply such explanations to genes like *armadillo,* which act early and are expressed in abundant quantities maternally and yet are also expressed zygotically.

Potentially more damaging to the model are genes that behave like the segment polarity gene *patched. patched* transcripts are not supplied maternally and zygotic transcripts accumulate in complicated patterns (NAKANO *et al.* 1989), consistent with a controlling role in development. However, no effects are observed when these patterns are altered by ectopic expression (INGHAM *et al.* 1991; SAMPEDRO and GUERRERO 1991). One possibility is that the informational content of the expression patterns is redundant. Various genes in the segment polarity subgroup are expressed in overlapping patterns and final cell fates are regulated by cell interactions that occur continuously throughout development. This means that patterned expression of a single component may contribute to the efficiency with which the final pattern is achieved, but does not function as an absolutely essential determinant of that pattern. Effects of ectopic expression may therefore be very subtle or transient.

In summary, limited expression patterns seem to play an important role in many of the gene activities that must be supplied to the embryo by zygotic transcription. In the majority of cases, gene products are transcribed in spatial patterns. In many cases where they have been tested, misexpression leads to at least transient abnormalities in development. This behavior is consistent with a role for their expression in controlling where and when specific developmental pathways are initiated. While real and significant exceptions exist, they are rare. In many of the exceptional cases it is not clear whether the analysis was sufficient to exclude subtle roles for the expression pattern, particularly when temporal as well as spatial aspects are considered.

How much is this extrapolatable to other species? In principle, one might expect similar selective pressures to maximize maternal contributions in all organisms that develop rapidly with little growth or increase in size before larval stages. Many organisms fit this description, including *Caenorhabditis elegans,* frogs, sea urchins and fish. The obvious exceptions are mammals and plants, both of which undergo substantial increases in size during early stages when spatial patterns are being established. The model would predict that the average gene in mammals or plants would show substantial transcriptional requirements in the embryo, because the increase in size makes it impractical to supply any gene product in sufficient quantities from purely maternal sources. In contrast to Drosophila, most random lethal mutations would cause death during embryogenesis; see JURGENS *et al.* (1991) and ROSSANT and HOPKINS (1992) for a discussion of this point. In the other organisms, transcription would still be required for only a few genes, but these would be predicted to play controlling roles in specific developmental processes.

It was the discrete phenotypes produced in sea urchin embryos when particular chromosomes were eliminated that led BOVERI to conclude that "the nuclear substance imposes its specific character on the developing trait." (In fact, had the early zygotic defects not been specific, BOVERI would not have been able to conclude that individual chromosomes carry distinct developmental properties.) His observations suggest that the model may apply to sea urchins and other marine organisms. A more concerted genetic approach is possible in other invertebrates, such as nematodes. In a survey of temperature-sensitive lethal mutations, HIRSH *et al.* (1978) identified 25 ts mutants affecting genes required for normal embryogenesis. The mutations had not been selected based on their morphological phenotypes and can be assumed to represent a random sample of gene activities required during that period. In 22 of the 25 cases, a wild-type allele in the mother was sufficient to rescue homozygous mutant embryos, arguing for the numerical predominance of maternal gene products in that organism. The three loci whose products must be supplied zygotically do not represent a very large sample; still, it would be interesting to know whether their zygotic phenotypes suggest specific roles in development. In any case, concerted mutagenesis screens for genes whose transcription is strictly required in *C. elegans* embryos should eventually identify all genes with specific morphological effects in that organism.

In vertebrates, less is known genetically about maternal *vs.* zygotic requirements. In frogs, maternal contributions appear to be substantial. Although few mutants are known, embryos homozygously deleted for ribosomal DNA develop to young tadpoles, even though new rRNA normally begins to be made at the blastula stage (BROWN and GURDON 1964). This indicates that rRNA and probably many cellular components are maternally supplied in quantities sufficient to last the duration of embryogenesis. Data for other vertebrates are not much more extensive. In principle, zebrafish has all the hallmarks of a species that should have minimal and therefore regulatory zygotic transcription. Development is rapid, the basic form being established in 48 hours (KIMMEL 1989). The volume of the embryo in-

creases only after hatching. Zygotically active mutations are known that significantly alter the pattern of the embryo. The real test will involve comparing the frequency of such mutations with the general behavior of random lethals. If the situation in zebrafish is like Drosophila, one would predict that only a small fraction of vital genes have transcripts that must be supplied to the embryo by its own transcription. Such genes should produce discrete phenotypes suggestive of specific roles in development. Results from the ongoing zebrafish screens (MULLINS *et al.* 1994; SOLNICA-KREZEL *et al.* 1994) will be interesting in this respect.

However, the major advantage of any mutagenesis screen is not the characterization of general behaviors of genes and their relative maternal and zygotic contributions. The real reason anyone does a screen is to identify interesting genes that offer entry points into studying complex phenomena. In the preceding pages, I have argued for the special advantage of screens directed at zygotic activity in the embryo. Under certain circumstances, those screens are particularly likely to identify controlling elements and will thus be particularly informative about how development is regulated. A second and unemphasized advantage of such screens is their simplicity, because phenotypes produced in early homozygous-mutant embryos are not complicated by later requirements for the same gene. Although chemical mutagenesis with point mutagens like EMS or ENU may only be possible in organisms where large numbers of stocks can be inbred over a number of generations, early acting, zygotically required genes can also be detected by deleting whole regions of the genome (as BOVERI showed in his classic paper). Because deletion embryos can be obtained in a reproducible fashion as meiotic segregants of crosses involving translocations, they can be generated in any organism where a substantial number of translocation chromosomes exist. This might allow an analysis of zygotic activity in mice, where many translocations are known and characterized, and surveys of transcriptional activity in any organisms where translocations can be isolated from natural populations.

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