tions and societies, and agencies funding, producing, and using research. A national body to monitor this information system should be constituted with federal and private participation.

I have not provided a "recipe" for how to bring this or whatever policy is correct into being. In truth, I am not even very optimistic. My recommendation number 6, for example, was in place in the 1950's and 1960's when the Committee on Scientific and Technical Information (COSATI) was in the Office of Science and Technology Policy. The executive coordinating function for science and technology information policy has been moved and downgraded ever since.

We will not make much progress in this area until the government takes a very pragmatic view of its whole science and technology policy—not just a data policy. I have long advocated such an overall policy, but I do it with a certain amount of concern.

The science and technology policy I suggest would expend federal funds on assuring that those solving problems in the interest of our society—whether they be in the public or private sector—have the best and most appropriate, most available technical means for doing so.

Now, if that were our federal science policy, the number one priority would be to make available existing knowledge. Most existing knowledge is very badly underutilized. If users benefited greatly from information services, there would be an increased demand for new knowledge to fill in the gaps—because massive gaps there are—and we would have a driving force for the basic research investment.

RESEARCH ARTICLE

Differential Gene Expression in the Gastrula of *Xenopus laevis*

Thomas D. Sargent and Igor B. Dawid

Summary. A modified cloning method designed to produce differential complementary DNA libraries permits the isolation of sequences that are present in the RNA population of any developmental stage or tissue, but are not present or are much less abundant in another stage or tissue. Selective complementary DNA cloning is especially useful when the differentially expressed RNA's are of low to moderate abundance in the cells in which they occur. A class of cytoplasmic polyadenylated RNA's differentially expressed in gastrula embryos of *Xenopus laevis* (DG RNA's) has been isolated. These DG RNA's occur very rarely or not at all in unfertilized eggs and blastulae, accumulate as the result of transcription before and during gastrulation, and, with some exceptions, decline in abundance as development proceeds. Many of these RNA molecules appear to be translated at the gastrula stage. Thus, DG RNA's may encode proteins that are important in the process of gastrulation.

One of the fundamental ideas of molecular embryology is that the process of development is controlled, directly or indirectly, by changes in the patterns of expression of nuclear genes. The spectrum of messenger RNA (mRNA) molecules synthesized, processed, and exported to the cytoplasm differs among tissue types, and in many cases is correlated directly, in the form of specific protein products, with a particular cellular phenotype. Many developmentally regulated genes whose expression is limited to certain terminally differentiated tissues or cell types have been isolated and studied (*1*). While much has been learned about eukaryotic gene expression from such work, genes whose expression is associated with the final stages of cellular differentiation seem unlikely to be involved in the initial processes of determination. Thus, it is of interest to isolate and study genes whose expression is regulated in the earliest stages of development, before any terminally differentiated tissues have appeared. In this article, we summarize the results of such a study.

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We chose as an experimental system the gastrula stage of *Xenopus laevis* because in this animal, as in others, gastrulation is the first event in embryogenesis that involves overt differentiation, leading to the elaboration of endoderm, mesoderm, and ectoderm (*2*). Furthermore, the eggs and embryos of *Xenopus* can be obtained easily, and considerable data on molecular aspects of genome organization, transcriptional, and translational activities and the composition of RNA populations in the embryos of this vertebrate are available (*3*). The unfertilized egg of *X. laevis*, like those of most animals, contains a sufficiently large supply of maternal mRNA to support protein synthesis at least through the blastula stage of development, and mechanisms apparently exist for selective utilization of various mRNA sequences (*4*). There is no detectable transcription of the *Xenopus* embryonic genome until after the 12th cleavage (the "midblastula transition") at which time transcription begins suddenly (*5*). Synthesis of pA+ RNA (*6*) proceeds at a rate sufficient to replace up to 30 percent of the maternal pA+ RNA mass by gastrula, with complete turnover attained as early as the neurula stage (*7*). While this synthetic activity demonstrates considerable gene activation in the early embryo, an earlier study has suggested that most of this newly synthesized RNA is homologous in sequence to RNA molecules present in the egg (*8*). Replacement of inherited RNA molecules with identical or similar transcripts rather than mobilization of previously unexpressed genetic information thus appears to be the primary function of early RNA synthesis in *Xenopus*. Nevertheless, the observation that interference with transcription during cleavage results in the arrest of gastrulation (*9*) suggests that some of the RNA sequences that are expressed by the gastrula are not included in maternal RNA. Such new RNA's would represent the earliest examples of developmentally

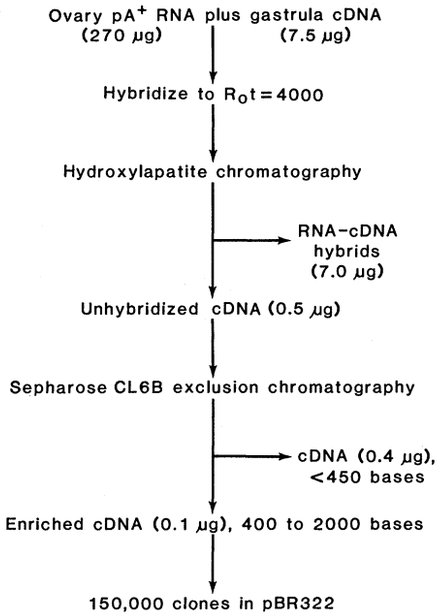


Fig. 1. Selective cloning of differentially expressed gastrula pA+ RNA sequences. RNA was purified by phenol extraction of postmitochondrial supernatants from disaggregated ovarian tissue or from embryos essentially as described (*25*). Polyadenylated RNA was purified by three cycles of oligo (dT) (deoxythymidylate) cellulose chromatography (*26*). Recovery of pA+ RNA averaged 0.3 to 0.5 percent of the total RNA for eggs, blastula, and gastrula, and 1.5 to 2 percent of the total RNA for tadpoles. Traces of DNA were removed from ovarian pA+ RNA to be used for hybridization by digestion with 5 μg of ribonuclease-free deoxyribonuclease I per 250 μg of RNA at 37°C for 30 minutes (*25*) followed by phenol-chloroform extraction and chromatography on Sephadex G100. High molecular weight cDNA for cloning purposes was synthesized from 15 μg of gastrula pA+ RNA essentially as described (*26*). Exhaustive hybridization of gastrula cDNA with ovarian pA+ RNA was carried out as follows. Ovarian pA+ RNA (270 μg) was mixed with 7.5 μg of gastrula cDNA, and hybridized to an equivalent $R_o t$ of approximately 4000 moles per liter per second (*27*). The sample was then applied to hydroxylapatite at 60°C, and single-stranded cDNA was eluted with 0.12*M* NaH_2PO_4, *p*H 6.8. This material was chromatographed on Sepharose CL6B, and excluded fractions were recovered. This cDNA was cloned into the Cla I site of pBR322 by G · C (G, guanine: C, cytosine) tailing (*26*).

regulated gene activity in the life cycle of this organism.

In principle, such sequences can be isolated by cloning of complementary DNA (cDNA) copies. Isolation of differentially expressed genes has been accomplished in other systems by screening recombinant DNA libraries with probes derived from various RNA populations and selection of clones that react preferentially with one or another probe (*10*). If the RNA populations being compared are very similar, a large number of clones must be screened to detect a significant fraction of the differentially expressed sequences. In practice, this requires colony or plaque hybridization, which is poorly suited for detecting RNA's that constitute less than about 0.05 percent. Since most animal cells contain 10,000 or more different cytoplasmic RNA sequences (*11*), most cellular mRNA's are too rare to be detected by such methods. To overcome this limitation, we have used a modified cDNA cloning procedure that provides a library highly enriched in the RNA sequences of interest.

Selective Cloning of Differentially Expressed Gastrula RNA's

Differentially expressed gastrula RNA's (DG RNA's) are defined as those present in gastrula cytoplasm but absent in the egg. These sequences can be purified by hybridization of gastrula cDNA to egg (or ovary) RNA and then cloning the cDNA that fails to hybridize.

The preparation of the DG library is summarized in Fig. 1. Gastrula cDNA was prepared from cytoplasmic pA+ RNA and exhaustively hybridized with an excess of ovary pA+ RNA. Under the conditions of this reaction (Fig. 1, legend), even sequences representing as little as 10^{-5} of egg pA+ RNA should hybridize to completion with homologous sequences in the gastrula cDNA (*12*). From the gastrula cDNA that failed to hybridize to egg pA+ RNA (6 to 7 percent), small molecules were removed and the remainder was inserted into the Cla I site of pBR322. The resulting enriched library contains more than 150,000 clones, many times the number of DG RNA sequences that are expected to exist.

The initial characterization of the DG library consisted of hybridization of radioactively labeled probes derived from pA+ RNA's from different developmental stages to filter-bound plasmid DNA of 84 individual DG clones [dot blots (*13*)]. Clones were selected at random, except that those that were either mitochondrial in origin or contained significant stretches of poly [d(A · T)] were excluded (*14*). Six clones (r1 to r6) from a reference gastrula cDNA library (*15*) were included as controls for probe integrity. Five background clones (b1 to b5) were also applied. The amount of hybridization was estimated by including M13 sequences in the probes at a level of 0.05 percent which reacted with M13 RF DNA applied to the filter as the last dot in the grid. Random-primer cDNA probes were prepared from cytoplasmic pA+ RNA extracted from unfertilized eggs, blastulae (stage 8 to 9), gastrulae (stage 10 to 11), and tadpoles (stage 41).

It is apparent that few, if any, of the DG library clones are represented at levels above background in either egg or blastula, and that approximately one-half increase significantly in abundance by

Table 1. Estimated abundance of RNA's in embryos. The mass of DG 42 RNA present in total gastrula RNA was measured by solution titration to be 48 pg. The mass per embryo of other DG RNA's and of r5 RNA at several stages was estimated by comparing hybridization levels of RNA dot blots by densitometry to that of DG 42 (Fig. 5). Corrections were made according to the following formula:

$$C_n = (L_r/L_i) \times M_r \times (D_n/D_{42}) \times 12$$

where C_n is the concentration in picograms per embryo of a particular RNA, L_r is the molecular weight of that RNA, L_i is the size of the cloned cDNA fragment, M_r is the total mass of embryonic RNA at the appropriate stage [taken as 4 μg for egg, blastula, and gastrula; 5 μg for late neurula; and 8 μg for tadpole (*30*)], D_n is the integrated densitometry scan of the appropriate RNA dot hybridization, and D_{42} is the corresponding value for DG 42 hybridized to gastrula total RNA. Estimated molecular sizes, in kilobases, are given for each RNA and for the cloned fragments of cDNA. N.D., not detected. The limit of detection in this experiment is approximately 2.5×10^{-7} of the RNA applied to the filter, that is, about 1/50 the intensity of DG 42 hybridized to total gastrula RNA.

Clone	Abundance (pg) per				Size	
	Egg	Gastrula	Neurula	Tadpole	RNA	Insert
DG 4	N.D.	5	N.D.	N.D.	2.3	0.7
DG 10	N.D.	15	5	5	5.4	1.2
DG 17	N.D.	9	3	1	3.1/2.4	1.6
DG 42	N.D.	48	13	N.D.	2.4	2.4
DG 56	N.D.	1	N.D.	N.D.	1.4	0.6
DG 70	N.D.	110	200	64	2.0	1.2
DG 81	N.D.	22	130	330	1.9	1.3
DG 83	N.D.	12	7	10	3.5	1.4
r5	22	28	69	290	2.8	2.2

the gastrula stage (Fig. 2). The control clones, r1 to r6, tend to hybridize to all stages to similar extents, although r1 behaves more like a DG RNA. Most of the RNA's that are positive in gastrula are not detectable in tadpoles by this method, although a few DG clones still hybridize to tadpole RNA, and one, DG 81, increases substantially. The total mass of pA+ RNA increases six- to tenfold per embryo between the gastrula and tadpole stages (*16*); most of this increase in mass is due to continued synthesis of sequences that are present in the egg (*17*). Therefore, the decline in fractional abundance of most DG RNA's from the gastrula to the tadpole stage implies that these RNA's are much less actively accumulated by the postgastrula embryo than the average pA+ RNA. This is in contrast to their behavior before and during gastrulation. The relative hybridization intensities of the DG clones and the M13 control suggest that DG RNA's range in abundance in gastrula from very low levels to a few tenths of a percent of pA+ RNA. As further shown below (Fig. 5 and Table 1) the dot blot hybridization allows identification of clones homologous to rare RNA's, that is, representing approximately 0.01 percent of the pA+ RNA population. Approximately one-half of the DG library clones do not react detectably with any of the four cDNA probes, although they all have inserts larger than 500 bp.

Characterization of Eight Clones from the DG Library

Eight DG clones, representing various gastrula abundances, and one clone from the gastrula reference library were selected for further analysis. To avoid choosing cDNA clones originating from a single RNA species, we undertook to cross-hybridize inserts from the clones most abundant in gastrula RNA. The results indicated that clones DG 8, 21, 40, 42, 45, 47, and 73, and clones 26 and 70 represent two redundant families. No other recurrences were detected. DNA from nine nonhomologous clones was labeled to uniform specific activity by nick translation and hybridized by the Southern technique to Eco RI-digested *Xenopus* genomic DNA (Fig. 3). Lane V was hybridized to a fragment of a vitellogenin cDNA clone (*18*); the intensity of hybridization in this lane thus represents what would be expected from a gene present twice per haploid genome. In the other lanes, the same DNA is hybridized to probes from the nine cDNA clones. Six clones, DG 4, 42, 56, 70, 81, and r5, are present once to a few times in the *Xenopus* genome. The complex pattern of hybridization to some of the low copy number clones is probably not due to partial cleavage by endonuclease in that the predicted partial cleavage bands for the vitellogenin gene do not appear in lane V (*18*). The multiplicity of bands may indicate (i) population polymorphism (DNA from five animals was pooled for these experiments), (ii) the presence of intervening sequences, (iii) multiple gene copies, or a combination of these factors. Clones DG 10, 17, and 83 hybridized to moderately repetitive *Xenopus* DNA. This is not entirely unexpected, since the presence of reiterated elements in the embryonic RNA populations has been reported (*19*). It is possible that some such elements would lead to cross-hybridization of different RNA's and consequently to removal of DG RNA's carrying repeated elements during the enrichment procedure. While some RNA's may have been lost in this manner, the presence in the DG library of clones hybridizing to reiterated DNA indicates that some repetitive families are not represented in the pA+ RNA pool until gastrulation.

When DG clones and r5 were hybridized to a gel blot of pA+ RNA prepared

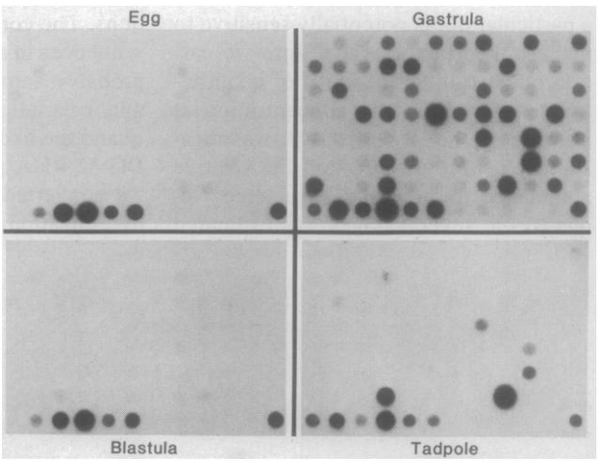


Fig. 2. Autoradiograms of DG plasmid DNA dot blots hybridized to labeled probes derived from pA+ RNA of eggs, blastulae (stage 8 to 9), gastrulae (stage 10 to 11) and tadpoles (stage 41). The four identical panels contain in their top seven rows arrays of 84 DG clones arranged in left-to-right, top-to-bottom order. Each bottom row contains, from left to right, six clones from a reference gastrula cDNA library, r1–r6 (*15*), followed by five background clones, b1–b5, which are Hae III fragments of pBR322 cloned into the Cla I site of pBR322 by G · C tailing. Each dot contains 1 μg of Bam HI–digested plasmid DNA that had been purified by cesium chloride–ethidium bromide centrifugation. The last dot is 1 μg of M13mp7 replicative form DNA, used as a hybridization control and abundance standard. Agarose gel electrophoresis of egg and embryonic pA+ RNA's revealed that all four preparations were essentially free of ribosomal RNA contamination and that all were similar in size distribution. Random-primer cDNA probes were prepared from these pA+ RNA's essentially as described (*28*) and supplemented with radioactive cDNA (final concentration 0.05 percent) prepared from M13 phage DNA. Hybridization background was reduced by hybridizing the cDNA with a large excess of DNA from a mixture of the background clones, b1–b5, followed by removal of double-stranded material, usually 10 to 15 percent of the cDNA, by hydroxylapatite chromatography. The filters were incubated first at 65°C for 1 hour in a solution of 0.1*M* NaH_2PO_4 (*p*H 6.8), 0.85*M* NaCl, 1 m*M* EDTA, 10× Denhardt's solution (*29*), 0.05 percent SDS, and 250 μg/ml each of wheat transfer RNA and denatured salmon DNA. Hybridization to radioactive probes was performed in a fresh volume of the same solution at 65°C for 12 to 24 hours. Filters were washed for 20 minutes in a mixture of 5× SSPE, 5× Denhardt's solution at 65°C, and then washed for 20 to 40 minutes in 0.2× SSPE at 65°C.

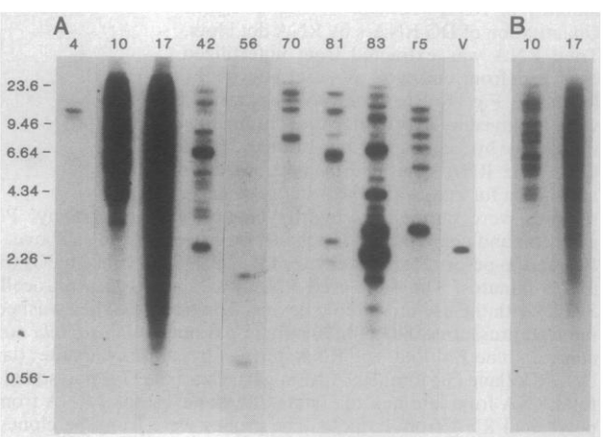


Fig. 3. Genomic Southern blots. *Xenopus laevis* nuclear DNA was prepared from erythrocytes pooled from five adult animals, and digested exhaustively with Eco RI and subjected to electrophoresis on a 0.8 percent agarose gel. Each lane is an identical blot of 5 μg of this DNA hybridized to a nick-translated restriction fragment containing the insert from the respective clone. The probe for lane V was a 1.1-kb Eco RI fragment from the vitellogenin cDNA clone, pX1vc 18 (*18*). Sizes are given in kilobases.

from whole gastrulae (Fig. 4), all clones hybridize primarily to a single well-defined species, except for DG 17, which reacts with two bands. Lane 42 is overexposed, and two higher molecular weight minor bands of hybridization are visible, perhaps representing nuclear precursor molecules.

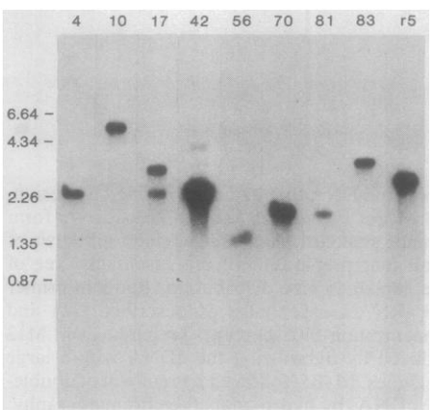
Estimated Abundance of DG RNA's in Total Embryonic RNA

While it is possible to estimate sequence abundance from the plasmid DNA dot blots (Fig. 2), this type of hybridization reaction is not terminated, and the amount of probe that is bound by a particular dot is potentially sensitive to uncontrolled factors. Therefore we carried out two different kinds of quantitation experiments: the concentration at gastrula of one RNA, DG 42, was measured by titration (*20*), and the relative abundance of the nine selected sequences at several stages was estimated by hybridization of the labeled cDNA clones to filter-bound RNA (RNA dot blots). For the titration, an excess of labeled single-stranded DNA probe prepared from DG 42 was hybridized with various amounts of gastrula RNA and the hybrids formed were assayed by S1 nuclease digestion (*20*). From these data we calculate that DG 42 represents a 1.2×10^{-5} fraction of gastrula RNA, or approximately 48 pg per gastrula.

The results of an RNA dot blot experiment are shown in Fig. 5. The intensity of hybridization to the various dots was measured by densitometry of suitable film exposures. These data allow reliable estimation of the relative abundances of one sequence in different RNA preparations. The comparison between different sequences in a given RNA preparation is probably somewhat less reliable due to minor variations in probe specific activity and the like. Taking the value of 48 pg DG 42 RNA per gastrula as a standard, we converted the relative intensities of hybridization in Fig. 5 into mass values for the nine RNA sequences at different stages. These values, corrected for the ratio of RNA length to insert length, are listed in Table 1.

These data indicate that all nine clones hybridize to varying but significant degrees to gastrula RNA; however, only the control, r5, shows any detectable homology to total egg RNA. Therefore, most if not all of the accumulation of DG RNA's is due to de novo transcription of previously silent genes, rather than to polyadenylation of preexisting nonpolyadenylated sequences. The results of Fig. 5 and Table 1 generally support and extend the data from Fig. 2 in showing that most DG sequences decline in abundance between gastrula and tadpole stages. The reference clone r5 increases tenfold, which is within the range of the increase shown by the total pA+ RNA population (*16*). Among the DG sequences, only DG 81 increases, and it does so more than the average pA+ RNA. Clone DG 83 remains approximately constant in mass per embryo and thus declines in fractional abundance, while the remaining DG RNA's decrease on a mass-per-embryo basis as well. This is particularly striking for DG 42, which is quite abundant at gastrula, has declined substantially by late neurula and is no longer detectable by the tadpole stage. The late neurula RNA dots tend to give intensities that are intermediate between the gastrula and tadpole values. An exception is DG 70, which appears to reach a peak sometime after gastrulation.

Transcription rates for particular cloned mRNA's can be estimated from the data shown in Table 1, if we assume that all nuclei in the embryo are actively synthesizing the particular RNA, beginning at the midblastula transition (*5*), and that the relevant gene is present once per haploid genome. According to the titration data, a gastrula contains 48 pg of DG 42 RNA, which represents 3.6×10^7 molecules. The estimated rate of transcription of the DG 42 gene, given the above assumptions, would be approximately nine completed transcripts per gene per minute (*21*). This rate is comparable to that reported for a maximally stimulated inducible gene such as vitellogenin (*22*). Of course, if DG 42 is expressed in a subset of the gastrula cells, this rate could be much higher in those cells, or if there is more than one active DG 42 gene per haploid genome, the transcription rate per gene would be correspondingly lower. Since there are several examples in the group of 84 DG clones with gastrula RNA abundances similar to that of DG 42, the implication



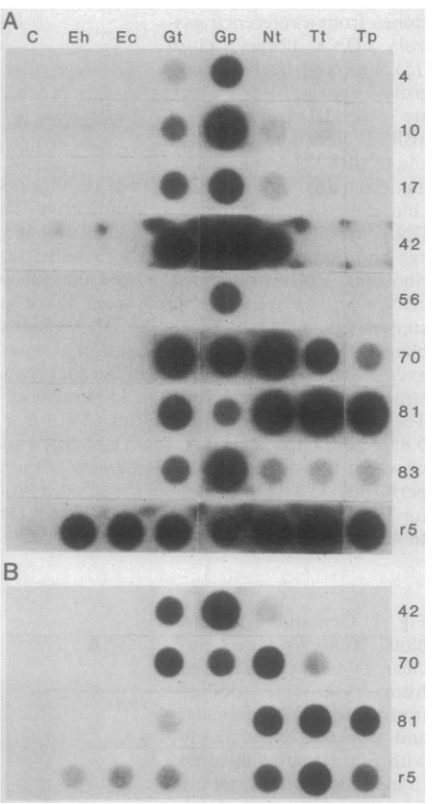


Fig. 4 (left). RNA gel blots. Each lane contains 0.5 μg of pA+ RNA purified from whole midgastrula embryos. The RNA was subjected to electrophoresis on a 1 percent agarose, 6 percent formaldehyde gel and transferred directly, without NaOH treatment, in 20× SSPE to a nitrocellulose membrane (*26*). Sizes in kilobases were inferred from the mobility of DNA markers. Fig. 5 (right). Quantitation of DG RNA's by RNA dot blots. Total RNA was extracted from unfertilized eggs and from embryos as described in the legend to Fig. 1. DNA and polysaccharides were removed by hydroxylapatite chromatography or by sedimentation through 5.7*M* CsCl. The RNA's purified by both methods are shown for egg, while the other total RNA samples were purified by hydroxylapatite chromatography. Polysomes were purified from gastrulae and tadpoles (*8*), and the RNA was extracted as above. RNA (3 μg) was suspended in 100 μl of 6 percent formaldehyde, 1*M* NaCl, 30 m*M* NaH_2PO_4 (*p*H 6.8), and incubated at 55°C for 15 minutes. The denatured RNA was applied to a nitrocellulose filter (saturated in 20× SSPE) with the use of Minifold device. The filter was then washed with 20× SSPE and baked at reduced pressure at 80°C for 90 minutes. (Lane C) *Drosophila melanogaster* RNA, background control; (lane Eh) total egg RNA purified by hydroxylapatite; (lane Ec) total egg RNA purified by CsCl; (lane Gt) total RNA from gastrula; (lane Gp) polysomal RNA from gastrula; (lane Nt) total RNA from late neurula (state 20); (lane Tt) total RNA from tadpole (stage 41); (lane Tp) polysomal RNA from tadpole. The probes were from the clones shown at right. Panel A was exposed for 100 hours and panel B for 10 hours.

is that several of the DG RNA's may be synthesized at extremely rapid rates, comparable to the most rapidly transcribed structural genes in differentiated tissues.

Figure 5 also includes dots of total polysomal RNA isolated from gastrulae and tadpoles. Since only approximately 15 percent of gastrula ribosomes are engaged in polysomal structures (*23*), a mRNA that is efficiently translated at that stage will be enriched by a factor of approximately six in total polysomal as compared to total cellular RNA. In contrast, nearly all ribosomes are found in polysomes in tadpoles, and consequently efficiently translated mRNA's should be equally abundant in total polysomal and total cellular RNA preparations from this stage. By these criteria, it can tentatively be concluded that DG 4, 10, 17, 42, 56, and 83 are efficiently translated at the gastrula stage, whereas DG 70, DG 81, and r5 are not; DG 70 and r5 appear to be poorly translated in tadpoles as well. DG 81 apparently exhibits the interesting property of being largely nonpolysomal in gastrulae and polysomal in tadpoles, although it is quite abundant in both stages. DG 81 is also the only example in the group of 84 clones which increases as a fraction of total pA+ RNA from gastrula to tadpole. This RNA, in contrast to the other DG RNA's examined, may participate in processes that occur later in embryogenesis.

Conclusions

In this report we describe the application of an enrichment cloning technique to the problem of gene expression in early development. A similar approach has been used to isolate RNA sequences differentially expressed in lymphoid cell lines (*24*), and is likely to be applicable to other developmental problems. A class of RNA molecules that is present in *Xenopus* gastrula but not in the maternal RNA pool is described. These differentially expressed gastrula RNA's accumulate in the cytoplasm as the result of vigorous transcription of nuclear genes, and most appear to be immediately translated on gastrula polysomes. It can thus be tentatively concluded that these gastrula RNA's are messenger sequences and that they correspond to proteins that appear before and during gastrulation. Furthermore, most RNA's in this class decline in abundance as development proceeds. This is particularly striking when compared to the behavior of the bulk of embryonic pA+ RNA, which increases six- to tenfold in mass per embryo by the tadpole stage. Therefore, the functions that are fulfilled by DG mRNA's and the proteins they encode are probably relevant to early development. It seems quite plausible that DG genes are important in the onset of differentiation that takes place at gastrulation, and elucidation of the functions of some of these genes should help in the understanding of the molecular basis of this process.

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6. Abbreviations used: bp, base pairs; nt, nucleotides; SDS, sodium dodecyl sulfate; SSPE, 0.18*M* NaCl, 10 m*M* NaH_2PO_4, *p*H 6.8, 1 m*M* EDTA; pA+, RNA molecules containing a 3′ polyadenylic acid moiety of sufficient length to cause binding to oligo deoxythymidylate (dT) cellulose; A, adenylic acid; T, thymidylic acid.
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$$D/D_o = \exp(-kR_o t)$$

See (*11*), p. 203.
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14. Clones containing either mitochondrial sequences or significant lengths of poly[d(A · T)] were identified by hybridization of nick-translated mitochondrial DNA or kinase-labeled poly(rA) (polyriboadenylate), respectively, to bacterial colony DNA bound to nitrocellulose [M. Grunstein and D. Hogness, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3961 (1975)]. Approximately 1 percent of the clones tested hybridized to mitochondrial DNA and 15 percent to poly(rA).
15. To provide a source of unselected gastrula pA+ sequences, a "reference" cDNA library was prepared by molecular cloning of cDNA synthesized from gastrula cytoplasmic RNA. Control clones were derived from this library by screening with probes made from egg and gastrula RNA's and choosing several that appeared to give strong signals with both probes.
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