Annu. Rev. Biochem. 1998. 67:335–94
Copyright © 1998 by Annual Reviews. All rights reserved

Metadata, citation and

uthors - Main

IN DEVELOPMENT

Arash Bashirullah,[1, 3] *Ramona L. Cooperstock,*[1, 2] *and Howard D. Lipshitz*[1, 2]

[1]Program in Developmental Biology, Research Institute, The Hospital for Sick Children, Toronto, Canada M5G 1X8; [2]Department of Molecular & Medical Genetics, University of Toronto, Toronto, Canada; [3]Division of Biology, California Institute of Technology, Pasadena, California; e-mail: lipshitz@sickkids.on.ca

KEY WORDS: *Drosophila*, *Xenopus*, *Saccharomyces*, oocyte, embryo, RNA-binding protein, cytoskeleton, microtubules, microfilaments, 3′-untranslated region (3′-UTR)

ABSTRACT

Cytoplasmic RNA localization is an evolutionarily ancient mechanism for producing cellular asymmetries. This review considers RNA localization in the context of animal development. Both mRNAs and non-protein-coding RNAs are localized in *Drosophila*, *Xenopus*, ascidian, zebrafish, and echinoderm oocytes and embryos, as well as in a variety of developing and differentiated polarized cells from yeast to mammals. Mechanisms used to transport and anchor RNAs in the cytoplasm include vectorial transport out of the nucleus, directed cytoplasmic transport in association with the cytoskeleton, and local entrapment at particular cytoplasmic sites. The majority of localized RNAs are targeted to particular cytoplasmic regions by *cis*-acting RNA elements; in mRNAs these are almost always in the 3′-untranslated region (UTR). A variety of *trans*-acting factors—many of them RNA-binding proteins—function in localization. Developmental functions of RNA localization have been defined in *Xenopus*, *Drosophila*, and *Saccharomyces cerevisiae*. In *Drosophila*, localized RNAs program the antero-posterior and dorso-ventral axes of the oocyte and embryo. In *Xenopus*, localized RNAs may function in mesoderm induction as well as in dorso-ventral axis specification. Localized RNAs also program asymmetric cell fates during *Drosophila* neurogenesis and yeast budding.

0066-4154/98/0701-0335$08.00

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

CONTENTS

INTRODUCTION . . . 336
PATTERNS OF RNA LOCALIZATION . . . 342
Drosophila Oocytes and Early Embryos . . . 342
Xenopus Oocytes . . . 348
Ascidian Oocytes . . . 352
Echinoderm Oocytes . . . 353
Zebrafish Embryos . . . 353
Polarized Somatic Cells . . . 353
MECHANISMS OF RNA LOCALIZATION . . . 355
Nucleo-Cytoplasmic Transport . . . 355
Transport from One Cell Type into Another . . . 355
Transport Out of Mitochondria . . . 356
Generalized Degradation with Localized Protection . . . 356
Directed Cytoplasmic Transport of RNA . . . 357
Entrapment/Anchoring of RNA at the Site of Localization . . . 362
RNA Transport/Anchoring Particles . . . 363
CIS-ACTING ELEMENTS THAT TARGET RNAs FOR LOCALIZATION . . . 364
Mapping of Cis-Acting Elements in Localized RNAs . . . 364
Cis-Acting Sequences for Localization Map to the 3′-Untranslated Region of mRNAs . . . 365
Cis-Acting Sequences for Localization Map Within Non-Protein-Coding RNAs . . . 367
Alternative Splicing Can Generate Localized vs Unlocalized RNA Isoforms . . . 367
Discrete Localization Elements . . . 368
Repeated/Redundant Localization Elements . . . 369
Dispersed/Nonredundant Localization Elements . . . 370
Additive Function of Localization Elements . . . 371
Elements That Function in Translational Control During or After Localization . . . 371
Primary, Secondary, Tertiary, and Quaternary Structures . . . 372
TRANS-ACTING FACTORS INVOLVED IN RNA LOCALIZATION AND TRANSLATIONAL CONTROL OF LOCALIZED RNAs . . . 373
Identification of Trans-Acting Factors . . . 373
Factors That Interact Directly with Defined RNA Elements . . . 374
Other Factors That Function in RNA Localization . . . 377
Summary . . . 382
DEVELOPMENTAL FUNCTIONS OF RNA LOCALIZATION . . . 383
Specification of the Anterior-Posterior and Dorsal-Ventral Axes of the Drosophila Oocyte . . . 383
Specification of Anterior Cell Fates in the Drosophila Embryo . . . 384
Specification of Abdominal Cell Fates in the Drosophila Embryo . . . 385
Assembly of Polar Granules and Specification of Germ Cells in the Drosophila Embryo . . . 386
Signaling of Dorso-Ventral Axis and Mesoderm Induction in the Xenopus Embryo . . . 386
Specification of Cell Fates During Asymmetric Cell Divisions . . . 387
EVOLUTIONARY CONSIDERATIONS . . . 388

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

INTRODUCTION

Since the early days of experimental embryology it has been suggested that the asymmetric distribution of substances in the egg cytoplasm might confer particular fates to cells that receive that cytoplasm (reviewed in 1). However, it is only in the past 13 years that specific maternally synthesized, asymmetrically distributed RNA and protein molecules have been identified in oocytes and

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

early embryos of *Xenopus*, *Drosophila*, ascidians, zebrafish, and echinoderms. This review focuses largely on RNAs that are localized to specific cytoplasmic regions in eggs and early embryos. It addresses both the mechanisms of cytoplasmic RNA localization and the developmental functions of this localization. Some consideration is also given to RNA localization later in development, in differentiating or differentiated cells. However, since both the mechanisms and the functions of this later localization are not well understood, the emphasis here is on RNA localization in oocytes.

This review considers only RNAs that are asymmetrically distributed in the cytoplasm. Examples of RNAs that are localized to and within the nucleus—even to specific chromosomes or regions of chromosomes (2–4)—are covered elsewhere. The first maternally synthesized cytoplasmically localized RNAs were identified in *Xenopus* in a molecular screen for RNAs enriched in either the vegetal (*Vg* RNAs) or animal (*An* RNAs) hemisphere of the *Xenopus* oocyte (5). Shortly thereafter, an RNA was discovered that is localized to the anterior pole of the *Drosophila* oocyte and early embryo (6). This RNA is encoded by the *bicoid* maternal effect locus (7), which plays a crucial role in specifying cell fates in the anterior half of the early *Drosophila* embryo (8). The facile combination of genetics and molecular biology in *Drosophila* led to *bicoid* becoming the first case in which it was demonstrated that RNA localization per se was important for normal development. Delocalization of *bicoid* RNA led to defects in anterior cell fate specification (7). Over 75 cytoplasmically localized RNAs have now been identified, and many of these are localized in eggs, early embryos, or differentiating cells (Table 1).

To date, it has been possible to address both the mechanisms and the developmental functions of RNA localization almost exclusively in *Drosophila* and *Xenopus*. The large size of the *Xenopus* oocyte has allowed mapping of sequences necessary and sufficient for RNA localization through injection of in vitro synthesized transcripts engineered to contain an exogenous reporter sequence and part or all of the localized RNA. Further, in some cases inactivation, delocalization, or degradation of specific RNAs has been induced through microinjection of antisense RNA or DNA. The ability to manipulate *Xenopus* oocytes and to apply various cytoskeleton-destabilizing drugs or other inhibitors has demonstrated the importance of the cytoskeleton in RNA localization.

Drosophila oocytes and early embryos are also large and have also been used for drug and inhibitor studies. In contrast to *Xenopus*, however, the ability to generate transgenic lines that express reporter-tagged transcripts during oogenesis has obviated the need for micronjection studies, although some of these have been conducted. Finally, the ability to obtain mutations in the endogenous gene that encodes the localized RNA or in factors that function in trans in its localization or in its translational regulation, has facilitated analyses of the

Table 1 Localized RNAs

Species	Transcript name	Protein product	Localization pattern	Cell	Reference
Ascidians					
	Actin	Cytoskeletal component	Myoplasm and ectoplasm	Oocyte	249
	PCNA	Auxilary protein of DNA polymerase	Ectoplasm	Oocyte	100
	Ribosomal protein L5	Ribosomal component	Myoplasm	Oocyte	102
	YC RNA	Noncoding RNA	Myoplasm	Oocyte	101
Drosophila					
	Add-hts	Cytoskeletal component	Anterior	Oocyte and embryo	16, 17
	Bicaudal-C	Signal transduction/ RNA-binding protein	Anterior	Oocyte	18
	Bicaudal-D	Cytoskeleton interacting protein (?)	Anterior	Oocyte	19
	bicoid	Transcription factor	Anterior	Oocyte and embryo	7, 32
	crumbs	Transmembrane protein	Apical	Cellular blastoderm	67
	Cyclin B	Cell cycle regulator	Posterior and perinuclear	Oocyte and embryo	59, 62
	egalitarian	Novel	Anterior	Oocyte	20
	even-skipped	Transcription factor	Apical	Cellular blastoderm	69
	fushi tarazu	Transcription factor	Apical	Cellular blastoderm	64
	germ cell-less	Nuclear pore associated protein	Posterior	Oocyte and embryo	57, 239
	gurken	Secreted growth factor	Anterior-dorsal	Oocyte	21
	hairy	Transcription factor	Apical	Cellular blastoderm	65
	Hsp83	Molecular chaperone	Posterior	Embryo	63
	inscuteable	Novel	Apical	Neuroblast	127
	K10	Novel	Anterior	Oocyte	22
	mtlrRNA	Noncoding RNA	Posterior	Oocyte and embryo	61, 134

(*Continued*)

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Table 1 (*Continued*)

Species	Transcript name	Protein product	Localization pattern	Cell	Reference
	nanos	RNA binding protein	Posterior	Oocyte and embryo	28, 43
	orb	RNA binding protein	Posterior	Oocyte and embryo	23
	oskar	Novel	Posterior	Oocyte and embryo	24, 25
	Pgc	Noncoding RNA	Posterior	Oocyte and embryo	26
	prospero	Transcription factor	Apical/basal	Neuroblast	127
	pumilio	RNA binding protein	Posterior	Embryo	250
	runt	Transcription factor	Apical	Cellular blastoderm	66
	sevenless	Transmembrane receptor	Apical	Eye imaginal Epithelial cells	126
	tudor	Novel	Posterior	Oocyte	27
	wingless	Secreted ligand	Apical	Cellular blastoderm	68
	yemanuclein-α	Transcription factor	Anterior	Oocyte	251
Echinoderms					
	SpCOUP-TF	Hormone receptor	Lateral to animal-vegetal axis	Oocyte	103
Mammals					
	β-actin	Cytoskeletal component	Specialized periphery	Fibroblasts, myoblasts, and epithelial cells	123–125
	Arc	Cytosketal component	Somatodendritic	Neurons	104
	BC-1	Noncoding RNA	Somatodentritic and axonal	Neurons	107, 111
	BC-200	Noncoding RNA	Somatodendritic	Neurons	108
	CaMKIIα	Signalling component	Somatodendritic	Neurons	109
	F1/GAP43	PKC substrate	Somatodendritic	Neurons	104
	InsP3 receptor	Integral membrane receptor	Somatodendritic	Neurons	110

(*Continued*)

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Table 1 (*Continued*)

Species	Transcript name	Protein product	Localization pattern	Cell	Reference
	MAP2	Cytosketal component	Somatodendritic	Neurons	106
	MBP	Membrane protein	Myelinating membrane	Oligodendrocyte and Schwann cells	120
	Myosin heavy chain	Cytoskeletal component	Peripheral	Muscle	252
	OMP/odorant receptors	Integral membrane receptor	Axonal	Neurons	118, 119
	Oxytocin	Neuropeptide	Axonal	Neurons	115
	Prodynorphin	Neuropeptide	Axonal	Neurons	116, 117
	RC3	PKC substrate	Somatoden-dritic	Neurons	104
	tau	Cytoskeletal component	Axon hillock	Neurons	112
	Tropomyosin-5	Cytoskeletal component	Pre-axonal pole	Neurons	113
	V-ATPase subunits	Membrane protein	Specialized membrane	Osteoclasts	122
	Vassopressin	Neuropeptide	Axonal	Neurons	253
Xenopus					
	Actin	Cytoskeletal component	Periplasmic	Oocyte	254
	Anl (a and b)	Cyoplasmic protein (ubiquitin-like)	Animal	Oocyte	5, 81
	An2	mt ATPase subunit	Animal	Oocyte	5, 82
	An3	RNA binding protein	Animal	Oocyte	5
	An4 (a and b)	Novel	Animal	Oocyte	83
	βTrCP	Signaling molecule	Animal	Oocyte	83
	βTrCP-2	Signaling molecule	Vegetal	Oocyte	83
	βTrCP-3	Signaling molecule	Vegetal	Oocyte	83
	B6	NR[a]	Vegetal	Oocyte	70
	B7	NR	Vegetal	Oocyte	70
	B9	NR	Vegetal	Oocyte	70
	B12	NR	Vegetal	Oocyte	70
	C10	NR	Vegetal	Oocyte	70

(*Continued*)

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Table 1 (*Continued*)

Species	Transcript name	Protein product	Localization pattern	Cell	Reference
	G-proteins	Signaling molecule	Animal	Oocyte	84
	Oct60	Transcription factor	Animal	Oocyte	85
	PKCα	Signaling molecule	Animal	Oocyte	86
	α-tubulin	Cytoskeletal component	Periplasmic	Oocyte	254
	VegT (*Antipodean*)	Transcription factor	Vegetal	Oocyte	91, 92
	Vgl	Signaling molecule	Vegetal	Oocyte	5
	Xcat-2	RNA-binding protein	Vegetal	Oocyte	78, 79
	Xcat-3	RNA-binding protein	Vegetal	Oocyte	95
	Xcat-4	NR	Vegetal	Oocyte	70
	xl-21	Transcription factor (?)	Animal	Oocyte	87
	Xlan4	P-rich and PEST sequences	Animal	Oocyte	88
	Xlcaax-1	Membrane protein	Animal	Oocyte	89
	Xlsirt	Noncoding RNA	Vegetal	Oocyte	97
	Xwnt-11	Secreted ligand	Vegetal	Oocyte	98
Yeast					
	ASH1	Transcription factor	Budding site	Mother cell	128, 129
Zebrafish					
	Vasa	RNA-binding protein	Cleavage plane	Early embryo	209

[a]NR, Not reported.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

mechanisms of RNA localization, the developmental functions of these RNAs, and of their localization per se.

This review begins with a description of patterns of cytoplasmic RNA localization with an emphasis on *Xenopus* and *Drosophila*. To help explain the patterns and their significance, brief descriptions of the structure and development of *Xenopus* and *Drosophila* oocytes and/or early embryos are included. After considering the patterns of RNA localization, the focus switches to mechanisms. First, the dynamics of RNA localization are considered, including the

role of the cytoskeleton in RNA transport and anchoring. Then specific components of the localization mechanism are dissected; these include *cis*-acting sequences and *trans*-acting factors that function either in localization per se or in control of RNA stability or translation during and after localization. Finally, developmental functions of RNA localization are discussed.

PATTERNS OF RNA LOCALIZATION

All cells are nonhomogeneous since they are compartmentalized into organelles with distinct functions and locations. These inhomogeneities can result in several forms of cellular symmetry and asymmetry. For example, positioning of the nucleus in the center of an otherwise quite homogeneous spherical cell produces spherical symmetry. In such a cell (and there are few if any examples, with the possible exception of some oocytes), certain RNAs might be localized close to the nucleus (perinuclear) while others might be positioned more peripherally. More complex cellular asymmetries result from variations in cell shape and the position of the nucleus and other subcellular organelles. Cells can be radially symmetric or even further polarized in two or three axes. In these cases, RNA localization can occur relative to one, two, or three axes (e.g. to the dorsal anterior pole). Regardless of cell shape or size, RNA distribution patterns are usually based on preexisting asymmetries and can, in turn, lead to the establishment of further asymmetries.

This section describes the dynamics and patterns of subcellular distribution of cytoplasmically localized RNAs. It provides a cellular and developmental context for consideration of the mechanisms and functions of RNA localization in subsequent sections. The emphasis here is on the best understood of the examples listed in Table 1.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Drosophila Oocytes and Early Embryos

STRUCTURE AND DEVELOPMENT OF THE NURSE CELL–OOCYTE COMPLEX

The two bilaterally symmetric *Drosophila* ovaries each consist of about 16 ovarioles. At the anterior tip of the ovariole is the germarium. Here the oogonial stem cells divide asymmetrically producing a stem cell and a commited cell, which is called a cystoblast. Each cystoblast divides four times with incomplete cytokinesis to form 16 cystocyte cells interconnected by cytoplasmic bridges that run through specialized membrane cytoskeletal structures called ring canals. Only 1 of the 16 cystocytes becomes the oocyte, and the remaining 15 become nurse cells. Each 16-cell germarial cyst becomes surrounded by somatically derived follicle cells to form a stage 1 egg chamber. The more posterior part of the ovariole comprises a connected series of progressively older egg chambers ordered such that the youngest is most anterior and the

oldest (stage 14) most posterior relative to the body axis of the female. It takes three days for an egg chamber to produce a mature egg. Except during the final six hours, the nurse cells synthesize large amounts of RNA and protein that are transported into the developing oocyte. Many of these molecules are required during the first two hours of embryonic development prior to the onset of zygotic transcription.

Selection of the oocyte from among the 16 cystocytes is not random. Of the 16 cells, 2 are connected to 4 others and 1 of these always becomes the oocyte (9). A large cytoplasmic structure—called the fusome—containing several cytoskeletal proteins, runs through the ring canals that connect cystocytes and has been implicated in oocyte determination (10–13). The only microtubule organizing center (MTOC) in the 16-cell complex is localized to the pro-oocyte, and microtubule arrays connect all 16 cells through the ring canals (reviewed in 14, 15). Because the MTOC nucleates the minus ends of the microtubules, the microtubule-based cytoskeleton that connects the 16 cells is polarized. This has important consequences for RNA transport from the nurse cells into the oocyte as well as for RNA localization within the oocyte itself.

RNA LOCALIZATION IN STAGE 1–6 EGG CHAMBERS Many RNAs that are later localized within the growing oocyte are first transcribed in all 16 germline cells but accumulate specifically in the pro-oocyte. These include the *Adducin like-huli tai shao* (*Add-hts*) (16, 17), *Bicaudal-C* (18), *Bicaudal-D* (19), *egalitarian* (20), *gurken* (21), *K10* (22), *orb* (23), *oskar* (24, 25), *Polar granule component* (*Pgc*) (26), and *tudor* (27) transcripts. Other RNAs that will later be localized within the oocyte are transcribed at very low levels in the nurse cells at this early stage and so cannot be visualized easily. For example, *nanos* is transcribed at low levels, and oocyte accumulation can be seen only later (28). Additional transcripts, such as *ovarian tumor* (29) and *cytoplasmic tropomyosin II* (*cTmII*) (30), also accumulate in the oocyte at this and later stages but are not localized. Therefore oocyte-specific accumulation is not unique to RNAs that will be localized within the oocyte during later stages of oogenesis but is a property of many RNAs synthesized in the germline of early egg chambers. The fact that many RNAs that accumulate in the early oocyte appear to do so with higher concentrations at the posterior cortex—the site of the only MTOC in the egg chamber (31)—is an early indication of the role of the polarized microtubule network in RNA transport and localization (see below).

The exact stage at which transcription of different localized RNAs commences (or at least the stage at which the transcripts can first be detected) varies. For example, *bicoid* is first transcribed in the nurse cells of stage 5 egg chambers and then accumulates in the oocyte (32), whereas *oskar* (24, 25) and *K10* (22) RNAs already accumulate in the oocyte in the germarium over a day

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

earlier. Interestingly, the dynamics of accumulation of these RNAs is identical if they are intentionally transcribed at the same time (33). Thus temporal differences in patterns of oocyte accumulation of different RNAs are a consequence of variation in time of transcription and are not indicative of a difference in underlying transport mechanism, which in fact is similar for different RNAs transcribed at distinct stages.

REORGANIZATION OF THE CYTOSKELETON AND RNA LOCALIZATION DURING STAGE 7 The process of nurse cell transcription and oocyte accumulation of RNAs continues through stage 6. During this time oocyte-follicle cell interactions establish anterior-posterior polarity within the oocyte and in the surrounding follicle cells (34, 35). Reciprocal signaling between the follicle cells and the oocyte results in a reorganization of the cytoskeleton such that, by the end of stage 7, the MTOC disappears from the posterior of the oocyte and microtubules become concentrated at the anterior oocyte margin (31, 34, 35). Concomitant with this change in cytoskeletal organization, RNAs that previously accumulated at the posterior of the oocyte localize in a ring-like pattern at the anterior oocyte margin (Figure 1) (e.g. *Bicaudal-C*, *Bicaudal-D*, *bicoid*, *egalitarian*, *gurken*, *K10*, *nanos*, *orb*, *oskar*, and *Pgc*) (18–26). At this stage several proteins are also seen in an anterior ring-like pattern (e.g. Egalitarian and Bicaudal-D) (20). This redistribution of RNA and protein is likely a consequence of the reorganization of the microtubule network such that the minus ends of microtubules move from the posterior pole to the anterior. Consistent with this, a β-galactosidase fusion to the minus-end-directed microtubule motor, Nod, relocalizes from the posterior pole of the oocyte at stage 6 to a ring around the anterior margin by the end of stage 7 (36). Thus RNAs that are transported into and within the oocyte by minus-end-directed microtubule motors would be expected to accumulate at the anterior rather than the posterior pole. As expected, the transient anterior localization of transcripts (e.g. *Bicaudal-D*, *bicoid*, *K10*, *orb*) is colchicine sensitive (37), and microtubules are required for anterior Egalitarian protein localization (20).

Mutations in genes involved in oocyte-follicle cell signaling during stages 6 and 7 cause defects in oocyte polarity (see below) and in the microtubule-based cytoskeleton (e.g. *Delta*, *gurken*, *Notch*, *PKA*) (34, 35, 38, 39). For example, double-anterior oocytes can form in which microtubules have their minus ends at both oocyte poles and their plus ends at its center. Mutations in *homeless* cause a similar disorganization of microtubules (40). Such disorganization results in *bicoid* RNA localization at both poles of the oocyte while *oskar* RNA and plus-end-directed kinesin-β-galactosidase fusion protein localize in the middle (38, 41, 42). These data emphasize that microtubule polarity directs intraoocyte transcript localization.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

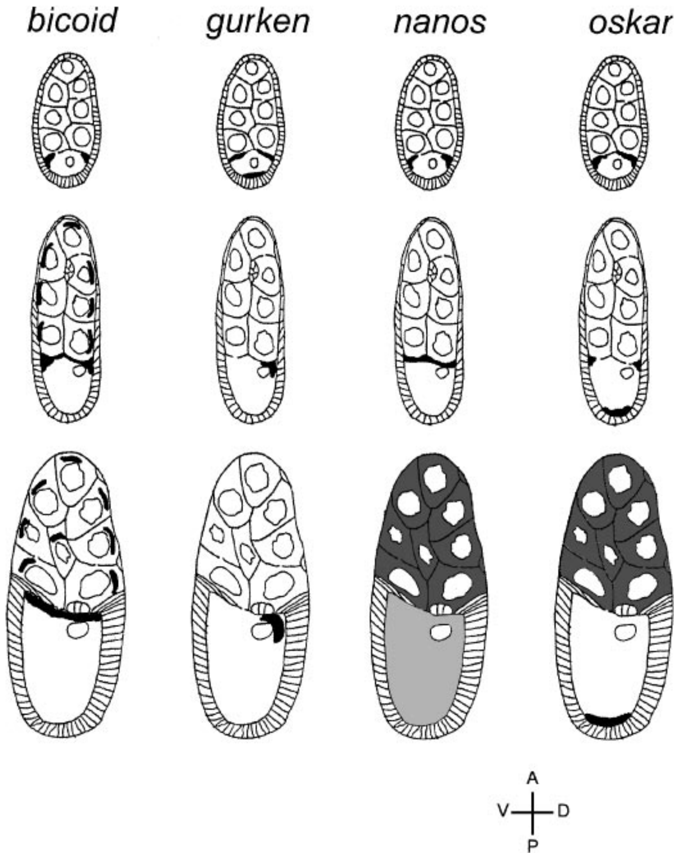

Figure 1 Localization of transcripts during *Drosophila* oogenesis. Transcript distribution patterns are shown in black or gray shading for *bicoid*, *gurken*, *nanos*, and *oskar* RNAs in stage 8 (*top row*), 9 (*middle row*), and 10B (*bottom row*) egg chambers. A, anterior; P, posterior; D, dorsal; V, ventral. Drawings of egg chambers are after King (9).

RNA LOCALIZATION AT STAGES 8–9 Further changes in RNA localization patterns occur at stages 8 and 9. Certain RNAs localize in a general anterior cortical pattern. These include *Bicaudal-C* (18), *Bicaudal-D* (19), *bicoid* (7), *K10* (22), *nanos* (28, 43), *orb* (23), and Pgc (26). In contrast, *Add-hts* RNA shifts from a general cortical pattern to an anterior cortical pattern (16, 44, 45; KL Whittaker, D Ding, WW Fisher, HD Lipshitz, manuscript in preparation) whereas *gurken* RNA localizes in a dorso-anterior pattern around the nucleus (21). Others such as *egalitarian* RNA delocalize from the anterior and become

uniformly distributed (20). Still others begin to relocalize to the posterior: *Pgc* RNA spreads posteriorly along the oocyte cortex (26) while *oskar* RNA gradually moves away from the anterior and begins to accumulate at the posterior pole of the oocyte (24, 25).

During this same period a kinesin-β-galactosidase fusion protein localizes to the posterior (41). This observation suggests that, even though it has not been possible to visualize microtubules that traverse antero-posterior axis of the oocyte at this stage (31), they must be present. RNAs that enter the oocyte at this stage or that were previously localized to the anterior of the oocyte in association with minus-end-directed microtubule motors, must dissociate from these motors and associate with plus-end-directed motors in order to be translocated to the posterior pole. One such RNA is *oskar*, which by the end of stage 9, is present only at the posterior pole of the oocyte (24, 25). *oskar* RNA plays a key role in nucleating formation of the polar granules at the posterior pole of the oocyte. The polar granules, which are involved in germ-cell specification (see below), gradually assemble during stages 9–14 of oogenesis (reviewed in 47).

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

NURSE CELL "DUMPING" AND RNA LOCALIZATION FROM STAGES 10–14 During stages 9 and 10 nurse cells increase synthesis of RNA and protein, dump their contents into the oocyte starting at stage 10B, and degenerate by the end of stage 12. This massive transfer of material is aided by contraction of the actin cortex of nurse cells. "Dumpless" mutants affect this process as well as ring canal structure. These genes, *chickadee* (encoding a profilin homolog), *singed* (encoding a fascin homolog), and *quail* (encoding a vilin homolog), are involved in F-actin crosslinking, indicating a major role for the actin-based cytoskeleton (48–50). Moreover, these studies demonstrate that the actin-based cytoskeleton is involved in anchoring the nurse cell nuclei so that they do not plug the ring canals during the dumping process. Interestingly, *bicoid* is localized apically in nurse cells during this phase (32). This apical distribution of *bicoid* RNA indicates a preexisting asymmetry within the nurse cells, but whether this transient *bicoid* localization in nurse cells serves any function is unclear. A similar apical nurse cell RNA localization pattern is observed for ectopically expressed *oskar* and *K10* transcripts at this stage (33).

During stage 10B, microtubules rearrange into subcortical parallel arrays in the oocyte, and a microtubule-based process called ooplasmic streaming begins. Capuccino and Spire proteins are required for control of ooplasmic streaming (51, 52). During stages 10B–12 the dumping of large amounts of RNA into the oocyte along with ooplasmic streaming make it difficult to distinguish delocalization of previously localized RNA on the one hand, from a transient stage during which the dumped RNA is becoming localized (i.e. is joining the

previously localized RNA at its intracellular target site) on the other. This is further complicated by the release of many transiently anteriorly localized RNAs, followed by their gradual translocation toward the posterior in some cases, or their complete delocalization in others. As a result, many RNAs appear to be generally distributed in the stage 11 oocyte. These include *Bicaudal-C* (18), *Bicaudal-D* (19), *egalitarian* (20), *nanos* (28), and *orb* (23). Other previously localized transcripts such as *K10* and *gurken* disappear by stage 11 (21, 22).

In contrast, the anterior localization pattern of *bicoid* and *Add-hts* transcripts is maintained throughout these stages (7, 16, 32). Maintenance of this pattern is likely the result of two factors: (*a*) anteriorly localized RNAs are trapped as they enter the oocyte from the nurse cells (WE Theurkauf, TI Hazelrigg, personal communication) and (*b*) previously anchored *bicoid* and *Add-hts* RNA is not released from the anterior pole during dumping and so does not have the opportunity to become generally distributed throughout the oocyte (16, 32).

After ooplasmic streaming is completed (stage 12), subcortical microtubules are replaced by randomly oriented short cytoplasmic filaments, and F-actin reorganizes from a dense cortical filament network to an extensive deep cytoplasmic network (31, 54, 55). At this stage, several newly localized RNAs can be seen at the posterior of the oocyte. These include *nanos* (28, 56), *germ cell-less* (57), and probably *orb* (23). In addition, *oskar* and *Pgc* transcripts exhibit a posteriorly enriched pattern (24–26). *bicoid* and *Add-hts* transcripts remain localized at the anterior pole of late oocytes (7, 16, 32).

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

RNA LOCALIZATION IN EARLY EMBRYOS After egg activation, the cytoskeleton reorganizes once again with actin and tubulin concentrated in the cortex and deeper filamentous networks of microtubules (31, 54, 55). Some longitudinal actin fibers may also be present in the early embryo. The *Drosophila* zygote undergoes 13 synchronous nuclear divisions without cytokinesis, forming a syncytial embryo containing several thousand nuclei that share the same cytoplasm (58). This syncytial state persists until the end of the 14th cell cycle when approximately 6000 nuclei reside at the cortex. Subsequently, invagination of membranes forms individual cells to give the cellular blastoderm. At this point the antero-posterior and dorso-ventral positional fates of the cells are specified.

The two anteriorly localized maternal RNAs—*bicoid* (Figures 1 and 2) and *Add-hts*—persist in early cleavage embryos. *Add-hts* is released and diffuses posteriorly (16), while *bicoid* appears to remain anchored at the anterior cortex (7, 32). By the cellular blastoderm stage both RNAs are gone.

Three RNAs that are posteriorly localized in the oocyte—*oskar*, *nanos* (Figures 1 and 2), and *Pgc*—retain posterior localization in early cleavage stage embryos. By nuclear cycle 6/7 *oskar* RNA is gone (24), whereas *Pgc* and

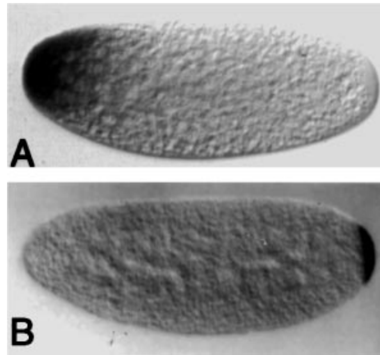

Figure 2 Localized maternal transcripts in early *Drosophila* embryos. *A*: *bicoid* RNA; *B*: *nanos* RNA. Images are of whole mount RNA tissue in situ hybridizations to stage 2 embryos using digoxigenin-labeled probes. Anterior is to the left and dorsal toward the top of the page.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

nanos RNAs are associated with polar granules and are taken up into pole cells together with these granules (26, 56). Some maternal RNAs do not become posteriorly localized until late in oogenesis or early embryogenesis. Examples are *Cyclin B* (59–62) and *Hsp83* (60, 61, 63) transcripts. By the cellular blastoderm stage, maternal transcripts of *Cyclin B*, *germ cell-less*, *Hsp83*, *nanos*, *orb*, and *Pgc* can be detected only in pole cells.

During the syncytial and cellular blastoderm stages, zygotic synthesis of RNA commences. Several of these zygotically synthesized transcripts, including *crumbs*, *even-skipped*, *fushi tarazu*, *hairy*, *runt*, and *wingless* are apically localized in the blastoderm (64–69), an epithelium surrounding the syncytial yolk mass of the zygote.

Xenopus Oocytes

As in *Drosophila*, maternally synthesized gene products play a key role in the development of the *Xenopus* embryo (reviewed in 70). Zygotic transcription initiates at the 4000-cell mid blastula stage. Unlike in *Drosophila*, however, synthesis of maternal molecules occurs in the oocyte itself. Thus the issue of transport into the oocyte from interconnected nurse cells does not arise.

DEVELOPMENT OF THE OOCYTE The *Xenopus* oocyte is initially a small spherical cell of 30 μm diameter when it is produced by mitosis of a stem cell, the oogonium (71). However, even at this stage, its nucleus and organelles are asymmetrically distributed (72, 73). Unlike *Drosophila* oogenesis, which lasts just over a week, *Xenopus* oogenesis lasts three years, although most of the

synthesis of oocyte contents occurs in the third year (71). The oocyte reaches a final diameter of approximately 1.5 mm (71).

An early indicator of asymmetry is the mitochondrial cloud or Balbiani body (71). It is composed of clumps of mitochondria, rough endoplasmic reticulum, and dense granules that initially are evenly distributed around the periphery of the germinal vesicle in early stage I oocytes (~80 μm diameter). By the end of stage I these components condense on one side of the germinal vesicle as a cap-like structure that grows and assumes a spherical shape. Beginning in stage II, the mitochondrial cloud moves toward the future vegetal pole initially changing shape to become disk-like and then reorganizing into a wedge-like shape (late stage II/early stage III). Subsequently its components become localized to the vegetal cortex of the oocyte during stage III/IV.

The mitochondrial cloud probably functions in the accumulation and localization of material needed for the formation of germ plasm at the vegetal pole of the early embryo (reviewed in 70, 74). In structure and function the *Xenopus* germ plasm is comparable to the *Drosophila* posterior polar plasm, and it contains germinal granules that function in germ-cell determination. However, while *Drosophila* polar granules are sufficient for the induction of germ cells (75, 76), *Xenopus* primordial germ cells are not irreversibly determined (77). Preliminary evidence indicates that certain RNA and protein components of the *Drosophila* and *Xenopus* germinal granules are evolutionarily conserved (70, 78, 79) and that in both cases RNA localization is an important mechanism used to locally assemble these structures.

The oocyte cytoskeleton is symmetric early in oogenesis. Until stage II the germinal vesicle appears to serve as the only MTOC with microtubules emanating radially toward the plasma membrane (80). This array loses its symmetry as the germinal vesicle moves toward the future animal pole and the mitochondrial cloud starts condensing at the opposite (vegetal) side. At this time microtubules start to concentrate at the vegetal side of the germinal vesicle, colocalizing with the wedge-shaped mitochondrial cloud by late stage II (77). The germinal vesicle completes movement to the animal hemisphere by stage V/VI and at this time the microtubule array disappears.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

RNA LOCALIZATION PATTERNS The first collection of localized RNAs was reported for *Xenopus* oocytes. Three animal hemisphere–enriched RNAs (*An1, An2, An3*) and one vegetal hemisphere–localized RNA (*Vg1*; Figures 3 and 4) were identified in a molecular screen for cDNAs representing mRNAs differentially distributed along the animal-vegetal axis (5).

The animal hemisphere (*An*)–enriched RNAs are not tightly localized within the animal hemisphere but are at least fourfold enriched in this hemisphere

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

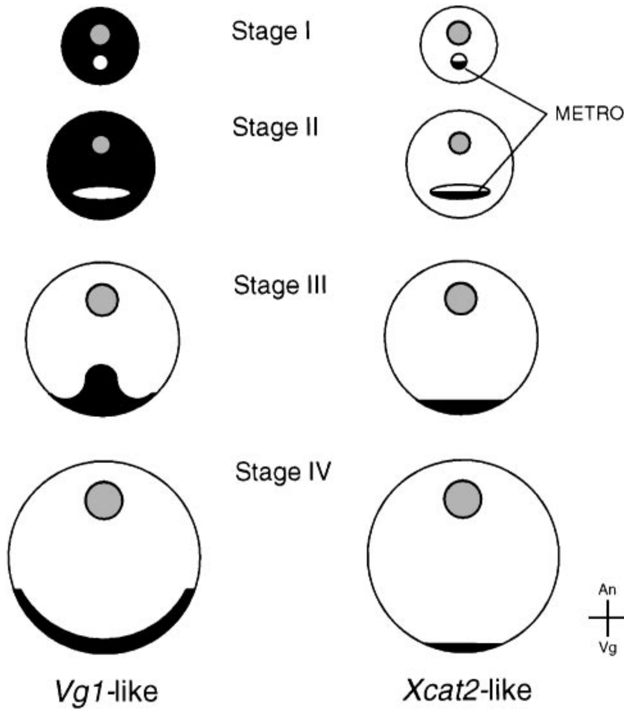

Figure 3 Localization of transcripts during *Xenopus* oogenesis. Transcript distribution patterns are shown in black, on the left for transcripts that show a *Vg1*-like pattern and on the right for transcripts that show an *Xcat-2*-like distribution pattern. Oocytes from stages I–IV are schematized. An, animal pole; Vg, vegetal pole. The germinal vesicle (oocyte nucleus) is shown in gray and the METRO as a white circle (stage I) or ellipsoid (stage II). Drawings of oocytes are after Kloc & Etkin (90).

relative to their vegetal concentration. Generally distributed maternal RNAs are twice as abundant in the animal than in the vegetal hemisphere (5). Thus the *An* RNAs are enriched in the animal hemisphere at least twofold versus other maternal RNAs. There are over a dozen known examples of *An* RNAs, all with similar distribution patterns: *An1a* (5, 81), *An1b* (5, 81), *An2* (5, 82), *An3* (5), *An4a* (83), *An4b* (83), *β-TrCP* (83), *G protein* (84), *oct60* (85), *PKC-α* (86), *xl-21* (87), *Xlan-4* (88), and *Xlcaax-1* (89). Unfortunately, reported in situ RNA hybridization data are lacking, making detailed comparisons of distribution patterns impossible.

In contrast to the *An* RNAs, the vegetally localized RNAs exhibit highly restricted distribution patterns. Careful observations of the patterns of localization

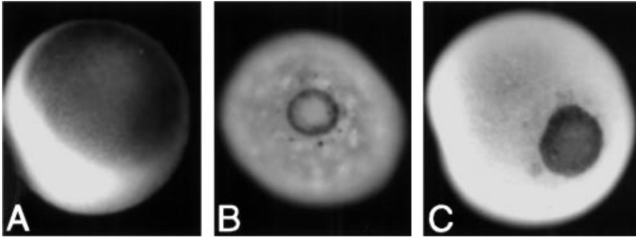

Figure 4 Localized transcripts in *Xenopus* oocytes. *A*: *Vg1* RNA; *B*: *Xcat-2* RNA; *C*: *Xlsirt* RNA. All images are of whole mount RNA tissue in situ hybridizations to either late stage III (*A*) or early stage II (*B, C*) oocytes. In *A*, the vegetal pole points toward the top right; in *B* and *C* the vegetal tip of the oocyte is at the center of the transcript distribution (i.e. the oocytes are viewed from the vegetal side). The small patches of *Xcat-2* RNA that are radially symmetrically distributed around the vegetal ring of transcripts are in the islands of germ plasm.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

in situ in whole mounts have suggested two or three distinct patterns (70, 90–92) exemplified by *Vg1*, *Xcat-2*, and, possibly, *VegT/Antipodean*.

Four RNAs show the *Vg1*-like pattern of localization: *Vg1* (Figures 3 and 4); and *Xcat-4*, *B12*, and *B9* (70). *Vg1* RNA is initially distributed uniformly in the oocyte cytoplasm and is excluded from the mitochondrial cloud during stages I and II (93). During late stage II/early stage III, *Vg1* transcripts accumulate in a wedge-like pattern toward the vegetal pole before becoming restricted to the vegetal cortex by late stage III (93–95). Finally, at stage IV, *Vg1* RNA is tightly localized to the cortex of the entire vegetal hemisphere (94, 96). This localization pattern correlates with the dispersion of the mitochondrial cloud (see above).

The second pattern of RNA localization is exhibited by seven RNAs: *Xcat-2*, *Xwnt-11*, *Xcat-3*, *B6, B7, C10,* and the *Xlsirts* (Figures 3 and 4) (70, 78, 79, 95, 97, 98). This localization process includes passage through what has been called a message transport organizer (METRO) within the mitochondrial cloud (Figure 3) and occurs in three distinct steps (90): (*a*) movement from the germinal vesicle to the mitochondrial cloud, (*b*) sorting within the METRO, and (*c*) translocation and anchoring to vegetal cortex. Localization begins during early oocyte stage I when transcripts appear to be distributed throughout the cytoplasm at low levels with slightly higher concentrations around the germinal vesicle. As the mitochondrial cloud condenses into a sphere during mid stage I, these transcripts are transported to the METRO. For *Xcat-2* this change in pattern is not the result of a reduction of RNA elsewhere, as the total amount of RNA in the oocyte remains constant or even increases (79). Within the METRO the transcripts are sorted such that, for example, *Xcat-2* is localized first, followed by *Xlsirts*, and then *Xwnt-11* (70, 79, 90, 99). By late stage I,

when the METRO is disc-like, *Xcat-2* RNA is in the periphery of the disc, *Xwnt-11* RNA is in the center, and *Xlsirts* reside between these two (Figure 3). The METRO moves vegetally such that by stage IV, all three RNAs are localized at the tip of the vegetal pole (70, 79, 90, 99). At this stage, the *Vg1* RNA pattern is quite distinct as it is distributed throughout the vegetal cortex (70, 79, 90, 99).

The third type of localization, typified by *VegT* (*Antipodean*), appears to deviate from both of these patterns, although there is some discrepancy in the distributions described in two reports (91, 92). *VegT/Apod* maternal transcripts are initially uniform in the cytoplasm in stage I. Later they appear to move to the vegetal pole with a timing and pattern similar to *Xlsirts*, *Xcat-2*, and *Xwnt-11* transcripts. One report describes a distribution at late stage III/early stage IV similar to that of *Vg1* (92), while another describes a novel final pattern predominantly in the vegetal yolk (91). The latter distribution would imply a target of localization that is distinct from all other vegetally localized transcripts.

After fertilization, the *B7* transcripts disappear, the *Xlsirt*, *Xcat-2*, and *Xcat-3* transcripts are associated with the germ plasm in the primordial germ cells, and the other transcripts (*B9, B12, C10, Vg1*, *Xcat-4*, and *Xwnt-11*) are all located in the vegetal blastomeres (70, 79, 90, 93, 98, 99; L Etkin, personal communication).

Ascidian Oocytes

Several RNAs are localized in ascidian (*Styela*) oocytes (Table 1). These include *YC* RNA, *PCNA* mRNA, and *ribosomal protein L5* mRNA. *PCNA* mRNA is initially uniformly distributed throughout the previtellogenic oocyte (100). During maturation, *PCNA* mRNA becomes concentrated in the central ectoplasm and cortical regions surrounding the myoplasm but is absent from the myoplasm per se (100). While *PCNA* mRNA can be observed in both the ectoplasm and the myoplasm after the first phase of ooplasmic segregation, which restricts the ectoplasm and myoplasm to the vegetal hemisphere, *PCNA* mRNA is absent from the myoplasm in the two-cell embryo (100). *YC* RNA is distributed throughout the cytoplasm of previtellogenic oocytes (101). However, during early vitellogenesis, *YC* transcripts are localized around the nucleus. They gradually move away from the nucleus as the oocyte increases in size, until they become restricted to the cortex of postvitellogenic oocytes (101). After the first phase of ooplasmic segregation shortly after fertilization, *YC* transcripts are localized in the vegetal cap of myoplasm (101). A third pattern of localization is exhibited by *L5* transcripts, which are concentrated in the cortical myoplasm (except at the animal pole) during oogenesis (102).

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

After fertilization, *L5* transcripts become restricted to the vegetal myoplasm (102).

Echinoderm Oocytes

There is one example of a maternally synthesized mRNA that is localized in the echinoderm (*Strongylocentrotus purpuratus*) oocyte and early embryo (103). This *SpCOUP-TF* mRNA, which encodes an "orphan" nuclear steroid hormone receptor, is localized subcortically in one hemisphere of the sea urchin oocyte and mature egg. Since there are no markers of the animal-vegetal axis of the egg, the location of *SpCOUP-TF* transcripts in the egg was inferred from their distribution in cleavage stage embryos where the animal-vegetal and oral-aboral axes are evident morphologically. From this inference it was concluded that *SpCOUP-TF* transcripts are localized such that they are restricted to one of the two cells produced by the first cleavage (i.e. lateral to the animal-vegetal axis) and, thus, are fixed at a 45° angle relative to the future oral-aboral axis (103).

Zebrafish Embryos

There is one example of a maternally synthesized mRNA that is localized within the cells of the zebrafish (*Brachydanio rerio*) embryo (209). This mRNA encodes a fish homolog of *Drosophila* Vasa, a DEAD-box RNA helicase that is known to function in germ plasm assembly (see below). Maternally synthesized zebrafish *vasa* transcripts localize to the inner (yolk-most) edges of the cleavage furrows at the first embryonic cell division (209). This localization pattern is maintained through the four-cell stage. From the 8- to the 1000-cell stage, the *vasa* transcripts remain in only four cells (the presumptive primordial germ cells) and are found in intracellular clumps that likely represent the assembling germ plasm. Subsequently, *vasa* transcripts are found in all primordial germ cells and germ cells.

Polarized Somatic Cells

Many cells in addition to oocytes are polarized. Epithelial cells have an apical-basal polarity. Differentiated neurons have dendritic arbors and an axon. Fibroblasts have specialized moving membranes (lamellopodia) at defined surfaces. These classes of cells also show asymmetric distributions of RNAs. However, in most cases, the developmental significance of RNA localization is unknown or, alternatively, transcript localization serves a function in the fully differentiated cell rather than during its development or differentiation. Several RNA localization patterns in these polarized cells are described here.

Although neurons can exhibit very complex and quite varied cytoarchitectures, they are classic examples of polarized cells and generally have an axon

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

on one side of the cell body (soma) and dendrites on the other. Most neuronal RNAs are present only in the soma and are excluded from dendrites and axons. At least 12 localized neuronal RNAs have been reported (1, 104, 105). These RNAs can be classified into two different patterns. The first is somatodendritic: *MAP2* (106); *BC-1* (107); *BC-200* (108); *CaMKII-α* (109); *IP3 receptor* (110); and *Arc*, *F1/GAP43*, and *RC3* (104) RNAs. The second is axonal: *BC-1* (111), *tau* (112), *tropomyosin-5* (113), *vasopressin/oxytocin* (114, 115), *prodynorphin* (116, 117), and *odorant receptor* (118, 119) RNAs. In almost all cases these RNAs are localized in differentiated neurons. The exception, *tropomyosin-5* (*Tm5*) RNA, is localized prior to any structural polarities at the future axonal pole of differentiating neurons (113). In mature neurons *Tm5* RNA is present only in the soma (113).

Several types of cells have defined areas of membrane devoted to a particular function. Myelinating membranes of oligodendrocytes and Schwann cells have associated myelin basic protein (MBP) mRNA (120, 121). mRNA for vacuolar H^+-ATPase subunits is localized to the bone resorption membranes of osteoclasts (122). Lamellopodia of fibroblasts (123) and myoblasts (124) contain localized cytoplasmic *β-actin* mRNA. Apical ends of villar epithelial cells also have high concentrations of *actin* mRNA (125). In these cases, the distribution of mRNA likely follows the differentiation of these cell types rather than playing a role during their differentiation.

In *Drosophila* the location of plus-end (kinesin)- and minus-end (Nod)-directed microtubule motors provides a readout of the polarities of various cell types. Localization of these motors within oocytes was mentioned earlier. In addition, these motors localize to opposite ends of polarized cells (36): epithelia (Nod is apical and kinesin is basal), mitotic spindles (Nod is at the poles), neurons (Nod is dendritic and kinesin is axonal), and muscle (Nod is at the center and kinesin at attachment sites). As mentioned previously, several mRNAs (e.g. *crumbs*, *even-skipped*, *fushi tarazu*, *hairy*, *runt*, and *wingless*) are apically localized within undifferentiated epithelia such as the embryonic blastoderm (64–69). In addition, mRNAs (e.g. *sevenless*) are localized within the developing epithelium of imaginal discs such as the eye disc (126).

It has been reported that *prospero* and *inscuteable* mRNAs are localized within embryonic *Drosophila* neuroblasts (127). The *inscuteable* transcripts are apically localized during interphase of the neuroblast cell divisions, while *prospero* transcripts are apically localized at interphase but are basal from prophase to telophase. Basal *prospero* RNA is segregated into one daughter cell (the ganglion mother cell).

The *S. cerevisiae ASH1* mRNA is localized within yeast cells at the site of the future bud and is segregated into the daughter cell (128, 129).

MECHANISMS OF RNA LOCALIZATION

This section focuses on general classes of RNA localization mechanisms. Specific details of *cis*-acting sequences and *trans*-acting factors that function in RNA localization are reviewed in the following section.

Nucleo-Cytoplasmic Transport

An obvious way to achieve cytoplasmic RNA localization is to export transcripts vectorially from only one side of the nucleus and then to transport or anchor them in the cytoplasm on that side of the nucleus. Substantial progress has been made recently in understanding the mechanisms of nucleo-cytoplasmic transport (130); however, studies of vectorial aspects of transport from the nucleus are in their infancy.

In general, it has been difficult to establish vectorial nucleo-cytoplasmic transport for particular transcripts due to experimental limitations. An exception is the case of pair-rule gene transcripts (*hairy* and *fushi tarazu*) in the cellularizing blastoderm of *Drosophila* (65, 131). Here it was possible to use mutations to produce two layers of nuclei (or displaced nuclei) in the cortex of the syncytial blastoderm and, thus, to show—for the inner nuclei—that transcripts are vectorially exported even in the absence of normal apical cytoskeletal structures. The fact that this is possible suggests that the nuclei themselves have a polarity independent of the cytoplasmic cytoskeleton. Moreover, this directed export depends on the 3′-UTR (65), suggesting that it is specific to these transcripts (see below for a discussion of the role of 3′-UTRs).

A second example of vectorial nucleo-cytoplasmic export may be the *Drosophila gurken* mRNA that is localized dorso-anterior to the nucleus in stage 8 oocytes. The *gurken* transcripts are synthesized in the oocyte nucleus itself (R Cohen, personal communication), and the K10 and Squid proteins may function in vectorial transport of the *gurken* mRNA (see below).

Transport from One Cell Type into Another

A second class of localization mechanism applies during *Drosophila* oogenesis. As outlined previously, RNAs are transported from the nurse cells into the oocyte through intercellular bridges known as ring canals. Nurse cells connect only to the presumptive anterior pole of the oocyte, so that the imported RNAs first arrive at the oocyte's anterior pole. It is likely that anteriorly localized RNAs (e.g. *bicoid*) are trapped at the anterior pole when they enter the oocyte (WE Theurkauf, TI Hazelrigg, personal communication). The fact that mutants in which nurse cells connect to the oocyte at both poles result in bipolar transport into the oocyte and trapping of *bicoid* RNA at both poles (42) supports this hypothesis. Recent experiments in which so-called localization particles were followed by time-lapse confocal microscopy supports this hypothesis further

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

(WE Theurkauf, TI Hazelrigg, personal communication). In contrast to the entrapment seen for anterior-localized RNAs, those that are localized to the posterior pole are actively transported there in association with the cytoskeleton or are localized there by other mechanisms such as degradation-protection (see below).

Transport Out of Mitochondria

The posterior polar plasm of *Drosophila* oocytes and early embryos contains large, non-membrane-bound organelles known as polar granules, which are involved in germ cell formation and specification (see below). Mitochondria are found in close association with the polar granules. One of the more remarkable examples of a localized RNA is the *Drosophila 16S mitochondrial large ribosomal RNA* (*mtlrRNA*), which is encoded by the mitochondrial genome (132). This RNA appears to be exported from the mitochondria into the cytoplasm within the posterior polar plasm and to be associated with polar granules (61, 133–137). Indeed, given the apposition of polar granules and mitochondria, the *mtlrRNA* may in fact be exported vectorially out of mitochondria directly into or onto the polar granules. The function of the *mtlrRNA* in the polar granules is unclear, although it has been implicated in pole cell formation (133). There is some disagreement, however, about whether a high local concentration of *mtlrRNA* is indeed necessary for pole cell formation (135–137).

Generalized Degradation with Localized Protection

It was postulated several years ago that one mechanism by which a generalized RNA distribution could be converted to a restricted pattern was through degradation of the RNA throughout the cell except at the site of localization (138). Several *Drosophila* transcripts represent variants of this type of process. For example, while the bulk of maternally synthesized *nanos* and *cyclin B* transcripts are concentrated in the posterior polar plasm of the early embryo, a subset of these transcripts remains unlocalized (62, 139–141). The posteriorly localized transcripts are taken up into the pole cells when they bud, while the unlocalized transcripts are degraded (62, 139–141). Similarly, maternally synthesized *Hsp83* transcripts are generally distributed in the early embryo (61, 63, 142; SR Halsell, A Bashirullah, RL Cooperstock, WW Fisher, A Karaiskakis, HD Lipshitz, manuscript in preparation). *Hsp83* transcripts in the posterior polar plasm also are taken up into the pole cells when they bud, while the remaining transcripts are degraded (61, 63, 142; SR Halsell, A Bashirullah, RL Cooperstock, WW Fisher, A Karaiskakis, HD Lipshitz, manuscript in preparation). There is a close correlation between translational repression of unlocalized *nanos* transcripts and their degradation (reviewed in 144). Under normal conditions, the polar granules are necessary and sufficient for protection of *nanos*, *cyclin B*, and *Hsp83* transcripts from degradation at the posterior (56, 60, 63).

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Another example of transcript destabilization and localization may be maternally synthesized *PCNA* mRNA in the ascidian oocyte. There is sequence complementarity between a non–protein-coding RNA, *YC*, whose 3′ end is complementary to the 3′-UTR of the *PCNA* RNA (100) as well as to the 5′-UTR of *ribosomal protein L5* RNA (Sc*YC26a*) (102). During oogenesis the *YC* RNA is perinuclear, gradually moving to the cortex, and after fertilization the RNA segregates to the myoplasm and associates with the cytoskeleton (101). Uniformly expressed maternal *PCNA* RNA initially overlaps with *YC* RNA but later becomes depleted in the myoplasm (100). Investigators have suggested that the double-stranded *YC-PCNA* RNA hybrid in the myoplasm might somehow destabilize *PCNA* RNA, thus representing an example of RNA localization by degradation-protection. The hypothesis that such double-stranded RNA hybrids destabilize specific RNAs in the myoplasm is, however, confounded by the data for the *L5 ribosomal protein* maternal RNA. Although there is substantial sequence complementarity between *YC* and *L5* RNAs, *L5* RNA is concentrated in myoplasm along with *YC* RNA, rather than destabilized there (102). In this case, the *YC* RNA has been postulated to aid the anchoring of *L5* RNA. Whether *PCNA* RNA localizes by a mechanism that involves hybrid-induced degradation remains an open question.

Directed Cytoplasmic Transport of RNA

Asymmetries in cytoskeletal organization have been described earlier for both *Xenopus* and *Drosophila* oocytes. Further, there is colocalization of specific RNAs with either a minus-end-directed microtubule motor (Nod) or a plus-end-directed motor (kinesin), in particular regions of the *Drosophila* oocyte's cytoplasm (see above). There is now substantial evidence that cytoplasmic RNA transport to specific intracellular destinations is accomplished by both the microtubule- and the microfilament-based cytoskeleton. The following section reviews evidence for a role of the cytoskeleton in anchoring localized RNAs at their intracellular destinations. Here the role of the cytoskeleton in directed cytoplasmic transport is reviewed.

Analysis of intracellular transport mechanisms requires the ability to systematically perturb normal cytoskeletal function. These studies have been aided in *Xenopus* and *Drosophila* by drugs that specifically perturb either the microtubule-based (colchicine, nocodazole, or taxol) or the microfilament-based (cytochalasins) cytoskeleton. In addition, mutations that affect components of the cytoskeleton have led to informative results in *Drosophila* and *Saccharomyces*.

THE ROLE OF THE CYTOSKELETON IN RNA TRANSPORT DURING DROSOPHILA OOGENESIS Localized RNAs have several characteristic and sequential patterns of expression during *Drosophila* oogenesis that correlate with particular aspects of the cytoskeleton, particularly the microtubules (see above).

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Over a dozen transcripts are synthesized in the nurse cells and specifically accumulate in the oocyte within early egg chambers prior to their localization [*bicoid* (7), *nanos* (28), *orb* (23), *oskar* (24, 25), *Add-hts* (16), *Bicaudal-C* (18), *Bicaudal-D* (19), *gurken* (21), *Pgc* (26), *K10* (22), *egalitarian* (20), and *tudor* (27)]. Transport of these RNAs into the oocyte is likely to be carried out by minus-end-directed microtubule motors since the MTOC is located in the oocyte during these stages (see above). Although no specific motors have been demonstrated to be involved in this process, the kinesin-like minus-end-directed motor—Nod—localizes first to the oocyte and then to its posterior at the same stages as many of these RNAs are transported into the oocyte and then accumulate at its posterior pole (36). Dynein (a minus-end-directed motor) is also localized to the oocyte at these stages (145) but does not appear to be involved in RNA transport and localization (146). During these stages, several RNAs are present in detergent insoluble fractions (e.g. *bicoid*, *oskar*, *Bicaudal-D*, *K10*, *orb*) indicating association with the cytoskeleton (147). Moreover, the association of *bicoid*, *oskar*, and *Bicaudal-D* RNAs with the cytoskeleton is sensitive to colchicine and not to cytochalasins, indicating that microtubules but not microfilaments are involved in their transport and localization (37, 147).

The phenotypes of *orb*, *egalitarian*, and *Bicaudal-D* mutants suggest a role in oocyte specific RNA accumulation. Egalitarian and Bicaudal-D proteins are made in nurse cells and are transported to the posterior of the oocyte presumably along minus-directed microtubules (20). The distribution of Egalitarian and Bicaudal-D proteins parallels that of the RNAs that are transported into the oocyte at these stages. Microtubule inhibitors result in delocalization of Egalitarian protein (20). Moreover, in *egalitarian* mutants *oskar* and *orb* RNA are no longer associated with the cytoskeleton (147). This indicates that Egalitarian and Bicaudal-D proteins may be involved—directly or indirectly—in transporting localized RNAs along microtubule networks into and to the posterior of the oocyte.

Mutations in genes required for early localization also perturb oocyte polarity; *egalitarian* and *Bicaudal-D* mutations cause all 16 cells of the cyst to become polyploid nurse cells; thus oocyte-specific accumulation of transcripts cannot occur because there is no oocyte (20, 148). Orb is required for oocyte polarity, and in *orb* mutants, oocytes are located at ectopic positions within the egg chamber (149). In *orb* mutant oocytes certain RNAs (*orb*, *oskar*) are still localized—albeit at abnormal positions—whereas others are not localized at all (*Add-hts*, *Bicaudal-D*, *K10*) (149). It is possible that Orb protein is required to establish microtubule polarity, whereas Egalitarian and Bicaudal-D are necessary for its maintenance.

As described above, the majority of RNAs transported into and localized in the oocyte have in common early transport from the nurse cells (stage 1–5),

transient localization to the posterior (stage 6), and subsequent localization to the anterior (stages 7–8). In addition, *oskar* and *Pgc* transcripts move back to the posterior pole of the oocyte at stage 9, whereas the *bicoid* and *Add-hts* RNAs never show the early posterior localization but are either always anteriorly localized (*bicoid*) or are initially localized throughout the cortex and subsequently localize to the anterior (*Add-hts*). These data suggest that a default transport and localization mechanism is carried out by minus-end-directed microtubule motors, and that certain RNAs (*bicoid*, *Add-hts*, *oskar*, *Pgc*) initially use this mechanism to enter the oocyte but then engage a different localization machinery. Since *bicoid* and *oskar* RNA transport and localization are best understood and exemplify distinct localization mechanisms, they are discussed below.

bicoid RNA is localized at the anterior pole of the oocyte by stage 8 of oogenesis and remains anterior until the late cleavage stage of embryogenesis when it is degraded. During its translocation from the nurse cells into the oocyte, it is apically localized within the nurse cells (32). Both apical nurse cell localization and anterior oocyte localization are sensitive to microtubule depolymerizing drugs (colchicine, nocodazole, tubulozole C) but not to inhibitors of F-actin polymerization (cytochalasin D and B) (150). Recent evidence suggests that transport of *bicoid* RNA into the oocyte actually involves several distinct steps that might be mediated by distinct localization mechanisms; for example, two distinct microtubule-dependent steps drive *bicoid* RNA localization particles within the nurse cell cytoplasm (WE Theurkauf, TI Hazelrigg, personal communication), but transport through the ring canals into the oocyte is resistant to both microtubule and actin filament inhibitors (WE Theurkauf, TI Hazelrigg, personal communication). The Exuperantia protein may mediate this microtubule-independent transport (151). The cytoskeletal association of *bicoid* transcripts is stage specific (147). During early oocyte accumulation, *bicoid* transcripts are associated with the cytoskeleton (i.e. the detergent-insoluble fraction). However, during stages 8–11, when *bicoid* transcripts are at the anterior margin of the oocyte, they are not cytoskeleton associated. This observation supports the idea that anchoring at the anterior pole at these stages is accomplished by some other structures. Later, during stage 14, *bicoid* RNA is localized in a tight cap at the anterior, is again associated with the microtubule-based cytoskeleton, and its localization is again sensitive to colchicine. In early embryos, *bicoid* RNA is no longer restricted to the cortex and is not associated with the cytoskeleton (147).

A kinesin-β-galactosidase fusion protein localizes to the posterior (41) at stages 8–9, indicating that microtubules traverse the antero-posterior axis of the oocyte at these stages and are oriented with their plus ends at the posterior (41). This localization coincides with the initiation of *oskar* and *Pgc* translocation

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

from the anterior pole of the oocyte to its posterior. Thus it is likely that *oskar* and *Pgc* switch from the use of minus-end-directed microtubule motors to the use of plus-end-directed ones in order to achieve transport to the posterior.

Posterior localization of the kinesin-β-galactosidase fusion protein is lost once ooplasmic streaming begins (stage 10) and is absent in *capuccino* and *spire* mutant oocytes that undergo premature streaming (41, 52). In *capuccino* and *spire* mutants *oskar* RNA is not localized to the posterior during stages 8 and 9, but instead *oskar* RNA is uniformly distributed throughout the oocyte (24, 25). These mutants cause an early cytoplasmic streaming during stage 7 and 8 instead of 10B (52), suggesting that premature assembly of microtubules into the parallel arrays in the subcortex drives cytoplasmic streaming (52). In other words, *oskar* RNA does not localize to the posterior in *capuccino* and *spire* mutants because these mutants omit the stage during which antero-posterior axial organization of microtubules is used for directed transport of *oskar* RNA to the posterior.

Evidence suggests a role for the actin-based cytoskeleton at the anterior of the oocyte in transfer of RNAs to the microtubules that run from the anterior to the posterior pole. In oocytes that are mutant for a component of the actin-based cytoskeleton—cytoplasmic (nonmuscle) tropomyosin II (cTmII)—*oskar* RNA remains anteriorly localized at stage 9 and never localizes posteriorly (152, 153). This observation suggests a role for cTmII—and possibly the actin-based cytoskeleton—in transfer of *oskar* RNA to the axial microtubules. Staufen protein similarly fails to translocate posteriorly in *cTmII* mutants (152), suggesting that the entire transport particle containing Staufen protein and *oskar* RNA fails to be transferred to the posterior translocation apparatus.

THE ROLE OF THE CYTOSKELETON IN RNA TRANSPORT DURING XENOPUS OOGENESIS As discussed above, the dynamics of transcript localization to the vegetal pole of *Xenopus* oocytes can largely be classified into two different patterns exemplified by *Vg1* and *Xcat-2* RNAs.

Xcat-2 transcripts are distributed uniformly in early *Xenopus* oocytes (79) and are then sequestered into the METRO region of the mitochondrial cloud along with *Xwnt-11*, *Xlsirt*, and other RNAs (70, 90). This step appears to be mediated by selective entrapment of these RNAs, possibly similar to posterior polar-granule-localized RNAs in *Drosophila* (see below). Once localized to the METRO, *Xcat-2* accompanies the mitochondrial cloud to the vegetal pole (90). *Xcat-2* RNA then relocates to form a disc-like pattern at the tip of the vegetal pole (90).

Vg1 RNA is initially generally distributed in the oocyte and later localizes in the wedge-shaped pattern that overlaps but differs from that of *Xcat-2* RNA at the vegetal pole (90). Accumulation of *Vg1* to the vegetal pole requires

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

functional microtubules but not actin microfilaments (94). Later *Vg1* RNA is found throughout the cortex of the vegetal hemisphere, unlike *Xcat-2*, which is localized to a more restricted area at the vegetal pole (90). During this late stage, *Vg1* RNA is enriched 30- to 50-fold in the detergent-insoluble fraction (96). Moreover, this association and cortical *Vg1* RNA localization are not sensitive to microtubule-depolymerizing drugs (nocodazole and colchicine) but rather to microfilament-disrupting agents (cytochalasin B) (94). This is an indication of a two-step localization mechanism for *Vg1* RNA where microtubules are required for translocation and actin filaments for anchoring (94). Recent evidence indicates that *Vg1* RNA is associated with the endoplasmic reticulum (ER) and that *Vg1* RNA-ER complexes move to the vegetal pole along with the mitochondrial cloud in a microtubule-dependent fashion (154).

Xcat-2 RNA injected into later oocytes is able to localize cortically without prior association with the METRO (99). This localization is dependent on microtubules and cannot occur in late oocytes (stage VI) when microtubules are no longer present (99). Moreover, the *cis*-acting elements within the *Xcat-2* 3′-UTR that are required for METRO localization and cortical localization are different but overlapping (79, 99) (see below). In addition, injected *Xcat-2* transcripts that localize to the vegetal cortex without METRO do so in a pattern similar to *Vg1* (throughout the vegetal hemisphere) but different from that of endogenous *Xcat-2* transcripts (99). Thus the differences in *Vg1* and *Xcat-2* localization patterns are a consequence of the fact that *Xcat-2* is normally associated with the METRO, rather than in some inherent difference in their ability to associate with the microtubule-based cytoskeleton.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

ROLE OF THE CYTOSKELETON IN RNA TRANSPORT IN OTHER CELL TYPES Observations of localized RNAs in living neurons in culture have suggested that they are present in particles composed of several RNAs and proteins including polyribosomes (155). These particles translocate inside the cell in a microtubule- but not microfilament-dependent manner (155). Similar studies with *MBP* RNA injected into oligodendrocytes in culture indicate that the initially homogeneous *MBP* RNA becomes organized into granules that align on microtubule tracks in the peripheral processes (156). Endogenous *MBP* RNA is seen in granules and fractionates with the insoluble fraction in cell extracts, consistent with an association of "transport" granules with the cytoskeleton (156).

Mammalian *tau* mRNA is localized to the proximal hillock of axons (112). This localization is mediated by the *tau* 3′-UTR (see below) and depends on microtubules (157). Interestingly, in vitro synthesized *tau* 3′-UTR injected into *Xenopus* oocytes localizes to the vegetal pole in a pattern identical to *Vg1* RNA (158). Moreover, this localization is dependent first on microtubules and then on

actin microfilaments, as for *Vg1* RNA (158). This observation demonstrates that once an RNA associates with the cytoskeletal transport apparatus, it localizes according to the type of cell in which it is. This occurs even if that RNA normally would not be present in this cell type. Thus the role of the cytoskeleton in RNA localization is highly conserved across evolution (see below).

RNA localization also occurs in the yeast *S. cerevisiae*. In this budding yeast, *ASH1* mRNA is localized first to the future bud site and then to the daughter cell by a mechanism involving actin microfilaments (128, 129). The role of microfilaments was demonstrated genetically using mutants in actin, myosin, profilin, and tropomyosin (which form part of the microfilament network). In contrast, disruption of microtubules by tubulin mutants, or disruption of the process of budding with *MYO2* mutants, has no effect on *ASH1* transcript localization.

In various somatic cells (e.g. fibroblasts and myoblasts) *β-actin* mRNA is localized to moving membranes (123, 124). This localization is not dependent on microtubules or intermediate filaments but on microfilaments (123). Both RNA transport and anchoring are dependent on the actin cytoskeleton (123).

The data described above indicate a key role for microtubules in directed mRNA transport, especially in *Drosophila* and *Xenopus* oocytes but also in polarized cells such as neurons and glia. In several cases, microfilaments also play a crucial role in RNA localization. However, it is unclear whether the instances of microfilament-based RNA localization indicate a role in directed RNA transport versus in entrapment/anchoring of the RNA at the site of localization (see below).

Entrapment/Anchoring of RNA at the Site of Localization

At stage 10B of *Drosophila* oogenesis, the microtubules in the oocyte assemble into subcortical arrays that direct circumferential ooplasmic streaming (see above). Thus at these stages, RNAs cannot be localized to the posterior by directed transport on axial microtubules. Localized RNAs most likely are trapped at the posterior as they circulate through the oocyte cortex along with unlocalized components of the cytoplasm. In support of this hypothesis, it has been shown that, while long-range transport of injected *oskar* RNA to the posterior requires microtubules (159), local injection of *oskar* RNA near the posterior pole of large (stage 10–11) oocytes or anywhere in smaller (stage 9) oocytes results in posterior localization in a microtubule-independent process. This observation suggests that short-range RNA transport and/or local entrapment of RNA is not dependent on microtubules. Trapping of injected *oskar* transcripts at the posterior pole fails in *cTmII* mutant oocytes (159), consistent with a role for the actin-based cytoskeleton in this process.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Egalitarian and Bicaudal-D may also be required for posterior anchoring or trapping of RNAs such as *oskar*. In a situation where Bicaudal-D is eliminated during late oogenesis (160), *oskar* RNA is initially localized normally but then is lost from the posterior pole. In these mutant oocytes kinesin-β-galactosidase protein localizes normally to the posterior; therefore, the microtubule-based cytoskeleton involved in posterior transport is not disrupted. Posterior RNA localization occurs independent of Bicaudal-D, but maintenance of localization is dependent on Bicaudal-D function. Bicaudal-D may be involved in anchoring of RNA at the posterior pole of the oocyte, where it may function in a complex with Egalitarian (20).

Anchoring of RNAs at the posterior pole of the *Drosophila* oocyte also requires the integrity of the polar granules. These are large non-membrane-bound organelles composed of RNA and protein. Many posteriorly localized RNAs are either components of the polar granules or are associated with the granules (reviewed in 142). Mutations that disrupt the posterior polar granules (e.g. *capuccino*, *oskar*, *spire*, *staufen*, *tudor*, *valois*, and *vasa*) cause delocalization of posteriorly localized RNAs while ectopic assembly of polar granules at the anterior pole of the oocyte results in anterior localization of RNAs normally localized to the posterior (reviewed in 142), with the possible exception of the *mtlrRNA* (137). Developmental functions of the posterior polar granules and their associated localized RNAs are considered below.

In *Xenopus* oocytes, anchoring of *Vg1* RNA to the vegetal cortex of stage IV oocytes requires microfilaments (94), which as for *Drosophila*, implicates the actin-based cytoskeleton in anchoring of RNA. Anchoring of *Vg1* RNA also involves other—non-protein-coding—localized RNAs, *Xlsirt*s (161). Injection of oligonucleotides complementary to *Xlsirt* transcripts causes delocalization of anchored *Vg1* RNA in a manner similar to delocalization by F-actin-disrupting drugs (161). This observation indicates a connection between *Xlsirt* RNA, *Vg1* RNA, and F-actin; however, no direct interactions have been demonstrated. A role for the noncoding *YC* RNA in anchoring *L5* RNA in the myoplasm of the ascidian oocyte and early embryo has also been postulated (102). However, no experimental evidence supports this hypothesis.

Finally, for *ASH1* RNA in yeast (128, 129) as well as for *β-actin* mRNA in mammalian somatic cells (123), the actin-based cytoskeleton is necessary for localization (see above). However, the mechanism of localization of these RNAs is not clear; it has not yet been determined whether localization is by selective entrapment or by directed transport.

RNA Transport/Anchoring Particles

Specific *trans*-acting factors that interact with localized RNAs are discussed later in a separate section. Large ribonucleoprotein RNA transport particles

have been visualized at the light microscope level in several systems. For example, *bicoid* RNA is transported from the nurse cells into the oocyte in particles that contain Exuperantia protein (162; WE Theurkauf, TI Hazelrigg, personal communication). Maintenance of *bicoid* transcripts at the anterior pole, as well as transport of *oskar* RNA to the posterior pole, likely involves particles that contain Staufen protein (163, 164). In *Xenopus*, RNA-containing transport particles have been visualized (90, 99). In neurons and glia, large transport particles containing *tau* RNA (157) and *MBP* RNA (156), respectively, have been identified. Particles containing *ASH1* mRNA have been detected in *S. cerevisiae* (129). The polar granules of *Drosophila* and the germinal granules of *Xenopus* are very large (organelle-sized) ribonucleoprotein (RNP) particles that are involved in anchoring localized RNAs at the posterior and vegetal poles of *Drosophila* and *Xenopus* oocytes, respectively. Several of the particles mentioned here contain not just proteins and mRNAs, but also non-protein-coding RNAs (26, 90, 97, 134, 155).

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

CIS-ACTING ELEMENTS THAT TARGET RNAs FOR LOCALIZATION

In principle, there are two mechanisms by which an RNA molecule could be targeted for cytoplasmic localization. An RNA element or elements could be recognized by the localization machinery. Alternatively (only in the case of mRNAs), the polypeptide product of the RNA might be recognized and the RNA translocated intracellularly along with the polypeptide. The latter mechanism—through recognition of the signal peptide by the SRP apparatus—may be used to bring mRNAs that encode secreted or transmembrane proteins into association with the endoplasmic reticulum (reviewed in 165). All other defined cytoplasmic RNA localization mechanisms involve recognition of RNA elements, particularly in the 3′-UTR of mRNAs. This section summarizes the methods used to map *cis*-acting sequence elements within localized RNAs. Generalities about the location of such elements within the RNAs are then drawn, and specific features of these elements are discussed. A subsequent section focuses on *trans*-acting factors that interact with these elements.

Mapping of Cis-Acting Elements in Localized RNAs

The general method used to map *cis*-acting sequences that function in RNA localization is to produce hybrid RNAs that include an exogenous reporter sequence (e.g. part of the *E. coli β-galactosidase* RNA) and part or all of the RNA under study. These hybrid transcripts are introduced into the cell type of interest, and the reporter sequence is used as a tag to assay localization of the hybrid transcript. Initially the location of the transcripts was assayed by isolating parts of cells and carrying out RNase protection assays for the presence of the

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

transcripts (166). However, this method was replaced by the use of in situ hybridization with antisense reporter RNA probes (e.g. antisense *β-galactosidase* RNA probes) either in tissue sections or whole mounts. More recently—in the case of injected in vitro transcribed RNAs that have been fluorescently tagged—it has been possible to visualize the injected RNAs directly by fluorescence microscopy (159). Elements involved in various aspects of localization are then mapped further by testing a series of RNAs that carry deletions or mutations in the region that confers localization. Using these methods, *cis*-acting sequences sufficient (i.e. that are capable of conferring localization) or necessary (i.e. that when deleted result in failure of localization) can be mapped. Additional information may be gained by performing sequence alignments of the region of interest among different species. Conserved domains may represent important *cis*-acting elements required for localization (167).

Methods available for introducing the hybrid RNA into the cell-type of interest vary depending on the system. In *Drosophila* transgenic flies can be obtained that synthesize the hybrid transcripts in the correct cell type and often at the correct developmental stage. This can be accomplished using an inducible promoter, a cell-type-specific promoter, or the promoter of the relevant endogenous gene. Thus there is seldom any question as to the in vivo significance of the results obtained. In many cases mutations exist that eliminate or reduce the function of the endogenous gene. This then enables one to test whether a transgene carrying all of the endogenous regulatory and protein coding sequences rescues the mutant phenotype. If so, then transgenes in which specific RNA elements are deleted can be assayed for phenotypic rescue or lack thereof; thus both the effects on RNA localization, and the phenotypic consequences of disrupting that localization, can be tested simultaneously.

A second common method for introducing the hybrid transcripts into the host cytoplasm is by injection of in vitro transcribed RNA. This has been done occasionally in *Drosophila* (60, 159) but is most common in *Xenopus* oocytes where transgenic technology is still rather primitive. In both *Drosophila* and *Xenopus*, the large size of the oocyte enables the injected RNA to be introduced at a specific location within the cell.

A third method, used mostly for analyses of somatic cells in culture, is to transfect expression plasmids carrying the hybrid transcription unit into the cells, to wait for transcription and possible localization to occur, and then to assay the distribution of the hybrid RNAs.

Cis-Acting Sequences for Localization Map to the 3′-Untranslated Region of mRNAs

Table 2 lists localized RNAs in which *cis*-acting localization elements have been mapped. For all mRNAs studied to date, sequences that are necessary for localization are found in the 3′-untranslated region (3′-UTR). In many cases,

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Table 2 *Cis*-acting localization elements

Species	Transcript name	Localization signal(s)	Sufficient subregions (subelements)	Reference
Drosophila				
	Add-hts	3′-UTR (345 nt)	345 nt sufficient (ALE1 = 150 nt)	—[a]
	bicoid	3′-UTR (817 nt)	625 nt sufficient (BLE1 = 53 nt)	166, 176
	Cyclin B	3′-UTR (776 nt)	94 nt + 97 nt (TCE = 39 nt)	182
	even-skipped	3′-UTR (190 nt)	163 nt (124 nt in UTR)	65
	fushi tarazu	3′-UTR (455 nt)	Not defined	65
	hairy	3′-UTR (816 nt)	Not defined	65
	Hsp83	3′-UTR (407 nt)	107 nt sufficient (protection)	—[b]
	K10	3′-UTR (1400 nt)	44 nt (TLS) sufficient	181
	nanos	3′-UTR (849 nt)	543 nt sufficient (localization) (TCE = 90 nt translational control) (SRE = 60 nt translational control)	43, 141, 185
	orb	3′-UTR (1200 nt)	280 nt sufficient	169
	oskar	3′-UTR (1043 nt)	924 nt sufficient (localization) (BRE = 71 nt translational control)	168, 184
	wingless	3′-UTR (1083 nt)	363 nt sufficient	—[c]
Mammals				
	β-actin	3′-UTR (591 nt)	54 nt or 43 nt sufficient (43 nt less active)	171
	BC1	5′ region (152 nt)	62 nt sufficient	173
	CaMKIIα	3′-UTR (3200 nt)	Not defined	109
	tau	3′-UTR (3847 nt)	1395 nt sufficient (VgRBP-binding region = 624 nt)	157, 158
Xenopus				
	Vgl	3′-UTR (1300 nt)	340 nt (VgLE) sufficient (85 nt repeat)	170, 183
	TGF β-5	3′-UTR (1102 nt)	Not defined	198
	Xcat-2	3′-UTR (410 nt)	150 nt (mitochondrial cloud) 120 nt (vegetal cortex)	99
	Xlsirt	3–12 repeat sequences (79–81 nt)	Two copies of repeat sufficient	97
Yeast				
	ASH1	3′-UTR	250 nt sufficient	128

[a]KL Whittaker, D Ding, WW Fisher, HD Lipshitz, manuscript in preparation.
[b]SR Halsell, A Bashirullah, RL Cooperstock, WW Fisher, A Karaiskakis, HD Lipshitz, manuscript in preparation.
[c]H Krause, personal communication.

these 3′-UTR elements are also sufficient for localization. Examples of mRNAs that contain such localization elements in *Drosophila* include anteriorly localized transcripts such as *bicoid*, *Add-hts*, and *K10* (22, 166; KL Whittaker, D Ding, WW Fisher, HD Lipshitz, manuscript in preparation), posteriorly localized transcripts such as *oskar*, *nanos*, *orb*, *Cyclin B*, and *Hsp83* (43, 60, 142, 168, 169; SR Halsell, A Bashirullah, RL Cooperstock, WW Fisher, A Karaiskakis, HD Lipshitz, manuscript in preparation), and apically localized pair-rule gene transcripts such as *even-skipped*, *fushi-tarazu*, *hairy*, and *wingless* (65). *Xenopus* also provides examples such as *Vg1*, *TGFβ-5*, and *Xcat-2* (99, 170). Interestingly, the rat *tau* RNA's 3′-UTR, which confers localization to the proximal hillock of rat axons, also mediates vegetal localization in *Xenopus* oocytes (158). The chicken *β-actin* 3′-UTR contains a *cis*-acting element sufficient for peripheral localization in chicken embryonic fibroblasts and myoblasts (171). The presence of localization tags in the 3′-UTR adds to the list of 3′-UTR *cis*-acting elements involved in posttranscriptional mRNA regulation via control of stability, cytoplasmic polyadenylation, and translation (172).

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Cis-Acting Sequences for Localization Map Within Non-Protein-Coding RNAs

Non-protein-coding RNAs also contain discrete elements that target the RNAs for localization (Table 2). Such elements have been mapped in *Xenopus Xlsirt* (97) and in neuronal *BC-1* RNAs (173).

Alternative Splicing Can Generate Localized vs Unlocalized RNA Isoforms

Drosophila Cyclin B transcripts exemplify the importance of alternative splicing of sequences that target localization (60). Alternative splicing within the 3′-UTR generates two *Cyclin B* mRNA isoforms that differ by 393 nucleotides (nt). The shorter splice variant is synthesized preferentially during early oogenesis and is present throughout the pro-oocyte until stages 7–8. The longer splice variant is synthesized in the nurse cells later in oogenesis, during stages 9–11. It is then transported into the oocyte, with an initially uniform distribution and is later concentrated at the posterior pole (60, 61). The transcript also exhibits perinuclear localization in the synctial embryo. Posterior localization of the long *Cyclin B* mRNA isoform is directed by the additional sequences spliced into its 3′-UTR relative to the short mRNA isoform (which is unable to localize) (60).

Alternative splicing also plays a role in the localization of *Add-hts* transcripts. Three classes of *Add-hts* contain unique 3′-UTRs introduced by alternative splicing (KL Whittaker, D Ding, WW Fisher, HD Lipshitz, manuscript in preparation). This alternative splicing also introduces variability in the

carboxy-terminal regions of the encoded Adducin-like protein isoforms (174; KL Whittaker, D Ding, WW Fisher, HD Lipshitz, manuscript in preparation). Only one of the mRNA variants, N4, exhibits transport into and localization within the oocyte (16; KL Whittaker, D Ding, WW Fisher, HD Lipshitz, manuscript in preparation). The N4 3′-UTR is necessary and sufficient for this transport and localization, suggesting that use of alternative 3′-UTRs is one mechanism by which different *Add-hts* protein isoforms are restricted to different subsets of the nurse cell–oocyte complex (KL Wittaker, D Ding, WW Fisher, HD Lipshitz, manuscript in preparation).

Although not a consequence of alternative slicing, two *actin* RNA isoforms, encoding α- and β-actin, possess isoform-specific 3′-UTRs that can confer differential intracellular targeting (175). *β-actin* transcripts are localized to the leading lamellae in both differentiating myoblasts and small myotubes, while *α-actin* transcripts associate with a perinuclear compartment.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Discrete Localization Elements

In the case of *bicoid* RNA, discrete elements within the 3′-UTR have been defined that confer distinct aspects of the RNA localization pattern. A decade ago a 625-nt subset of the *bicoid* 3′-UTR was found to be sufficient for anterior localization (166). At that time it was suggested that the secondary structure of the 3′-UTR, which can be folded into several long stem-loops, might be recognized by the localization machinery. Subsequent evolutionary sequence comparisons supported this hypothesis since the secondary structure appears to be conserved in distant *Drosophila* species (*melanogaster*, *teissieri*, and *virilis*) despite the fact that the primary sequence of these 3′-UTRs has diverged by up to 50% (167). Further, the 3′-UTR from one species can direct anterior localization in a distant species (167). There is complementarity between two single-stranded regions predicted in the secondary structure, implying that tertiary base-pairing interactions might also be important (167).

Subsequent analyses followed two different routes. In one set of experiments, deletions were used to define an approximately 50-nt region, called *bicoid* localization element 1 (BLE1), which is necessary and sufficient (when present in two copies) to direct nurse cell–oocyte transport and anterior transcript localization during mid-oogenesis (176). However, anterior localization is lost later. BLE1 interacts with Exl protein, which might function in localization to the anterior of the oocyte (177) (see below). In addition, linker scanning and point mutational analyses were used to define regions of the 3′-UTR that are important for anterior localization late in oogenesis and in the early embryo (163, 164). These regions interact with the double-stranded RNA-binding protein Staufen (178, 179), which functions to anchor *bicoid* RNA at the anterior of the late-stage oocyte and early embryo (see below). This is accomplished in

part by promoting quaternary (inter-3′-UTR) interactions via the complementary single-stranded regions mentioned earlier (164).

Although not yet as well characterized as *bicoid*, it has similarly been possible to map discrete elements in other 3′-UTRs that direct subsets of the localization pattern. For example, the N4 isoform of *Add-hts* mRNA is transported from the nurse cells into the oocyte starting in the germarium, then localized cortically in the oocyte (stages 7–8) and to the anterior pole (stage 9) (16, 44, 45, 180). In this case, a central (100–150 nt) element of the 3′-UTR, *Add-hts* localization element 1 (ALE1), is necessary and sufficient for nurse cell–oocyte transport as well as for cortical localization within the oocyte (KL Whittaker, D Ding, WW Fisher, HD Lipshitz, manuscript in preparation). The region that includes ALE1 comprises the most extensive predicted secondary structure within the *Add-hts* N4 3′-UTR (KL Whittaker, D Ding, WW Fisher, HD Lipshitz, manuscript in preparation). When additional, adjacent parts of the N4-3′-UTR are added to ALE1, anterior localization is conferred starting at stage 9 (KL Whittaker, D Ding, WW Fisher, HD Lipshitz, manuscript in preparation). The *K10* 3′-UTR reveals several long inverted repeats, suggesting that it forms extensive secondary structure (22). A short region within the *K10* 3′-UTR, TLS (transport/localization sequence, 44 nt in length) is predicted to form a stem-loop structure and is necessary and sufficient for nurse cell–oocyte transport and anterior localization (181). Mutations that disrupt this structure block transport and localization, while compensatory mutations that preserve the structure restore these processes.

With respect to posteriorly localized RNAs, it has also been possible in some cases to map discrete localization elements. For example, as mentioned above, a 181-nt element in the long isoform of *Cyclin B* mRNA is necessary for posterior localization (182). Similarly, a 107-nt element in the *Hsp83* 3′-UTR is necessary and sufficient for association with the posterior polar plasm (SR Halsell, A Bashirullah, RL Cooperstock, WW Fisher, A Karaiskakis, HD Lipshitz, manuscript in preparation).

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Repeated/Redundant Localization Elements

In the examples described above it was possible to define discrete, relatively small (<150 nt) localization elements that confer specific aspects of localization. In other instances, while discrete elements have been identified that direct localization, there is some redundancy in the system such that more than one localization element capable of conferring a particular aspect of the localization pattern, is present in the RNA.

An example comes from the *Xenopus Vg1* 3′-UTR. A 340-nt region within the 3′-UTR is necessary and sufficient for vegetal localization of *Vg1* RNA (170). Deletion analysis indicates that there is considerable redundancy within

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

this region but that critical elements can be defined that lie at each end (183). An 85-nt subelement from the 5′ end of the region, when duplicated, is sufficient to direct vegetal localization (183).

A second example is in the *orb* 3′-UTR (169). In early oogenesis, *orb* transcripts accumulate preferentially in the pro-oocyte (stage 1). They localize transiently to the oocyte posterior (stages 2–7) and then to the oocyte anterior (stages 8–10). A 280-nt element is sufficient to confer oocyte accumulation, posterior localization, and then anterior localization. Further analysis has shown that when the element is split in two, each half on its own confers oocyte accumulation, although the level of accumulation is reduced relative to the intact element (169). Several possibilities could account for this result. Each element may constitute an independent binding site for the localization machinery, and the presence of both elements might recruit more localization factors. Alternatively, the two elements may interact with each other to present a better binding site and recruit a single binding factor.

A third example comes from analysis of the chicken *β-actin* 3′-UTR (171). *β-actin* mRNA is localized to the leading edge of lamellae in chicken embryonic fibroblasts and myoblasts. A so-called peripheral zipcode element consisting of the first 54 nt of the 3′-UTR is sufficient to direct localization of a heterologous transcript. When this element is deleted from the full-length 3′-UTR, the transcript is still able to localize, suggesting the presence of a redundant element. An inspection of the remainder of the 3′-UTR revealed a region of homology to the 54-nt zipcode within a more 3′-located 43-nt sequence. When this 43-nt region is present on its own, it is able to direct localization, albeit less effectively than the 54-nt element. These data suggest functional redundancy, but a functional analysis gave complicated results. Transfection of oligonucleotides complementary to each element individually significantly reduced localization (171). This observation may suggest that both elements are required in their natural context to mediate localization and, therefore, that they are not fully redundant. However, each element can mediate localization in isolation. In addition, antisense oligonucleotides used may have recognized both elements (since they share sequence homology) and thus simultaneously inactivated both localization elements.

A final case of repetition comes from the noncoding *Xlsirt*s. These RNAs include repeated sequence elements flanked by unique sequences. The repeated element is 79–81 nt long and is tandemly repeated 3–13 times (97). Vegetal localization can be conferred by as few as two of the 79-nt sequence elements (97).

Dispersed/Nonredundant Localization Elements

In contrast to the aforementioned examples, in some cases it has been difficult to map discrete localization elements. For example, the *nanos* 3′-UTR contains

a 547-nt region that is necessary and sufficient to confer localization (43). Two overlapping subregions map within this larger region, either of which are capable of conferring localization. However, these subregions are 400 and 470 nt in length, respectively, and cannot be subdivided without disrupting localization (43). Another possible example is the 1043-nt *oskar* 3′-UTR (168). In this case deletions have been used to define elements necessary for distinct aspects of *oskar* RNA localization. However, it has been difficult to demonstrate sufficiency of individual elements for any specific aspect of localization (168).

In principle, several scenarios might prevent definition of discrete and small (<150 nt) elements sufficient for localization. For example, there might be several dispersed elements, each necessary for localization, distributed over a large region. Deletion of any one of these elements would disrupt localization. Alternatively, the arrangement of specific localization elements within the larger region or the secondary structure of the RNA might preclude the use of gross deletional studies to define subregions sufficient for localization.

Additive Function of Localization Elements

Implicit in much of the preceding discussion is the fact that different subsets of the RNA confer different aspects of the localization pattern. In other words, localization elements act additively. Examples already mentioned are the *bicoid* 3′-UTR, which has distinct elements for early (BLE1) vs later (Staufen-mediated) localization to the anterior pole, and the *Add-hts* N4 3′-UTR, which has an early transport and cortical localization element (ALE1) and distinct elements that function in anterior localization. In each case, the combination of the defined elements (with possible contributions from other undefined elements) directs localization with the correct spatial and temporal dynamics.

Elements That Function in Translational Control During or After Localization

Several localized RNAs in the *Drosophila* oocyte are not translated until they are localized. For example, *oskar* RNA is not translated until it is localized to the posterior pole of the stage 9 oocyte. Translation of unlocalized *oskar* RNA leads to major developmental defects. Thus there is an intimate and important link between localization and translational control of *oskar* RNA. Sequence elements for translational control are separable from those that function in localization per se. Elements known as Bruno response elements (BREs) have been mapped within the *oskar* 3′-UTR and are necessary and sufficient for preventing translation of unlocalized RNA. Three discrete segments (A, B, and C) within the 3′-UTR bind an 80-kDa protein (Bruno) that mediates translational repression (discussed in the next section) (184). These segments share a conserved 7- to 9-nt sequence [U(G/A)U(A/G)U(G/A)U] that is present as a

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

single copy in elements A and B, and in two copies in element C. Mutation of this sequence abolishes binding of Bruno and thus translational repression of unlocalized *oskar* RNA (184).

A second example comes from the *nanos* RNA. Unlike *oskar* RNA, which becomes tightly localized to the posterior pole of the oocyte, some unlocalized *nanos* RNA always exists in the embryo even after most of it is localized to the posterior. This unlocalized *nanos* RNA must be translationally repressed in order to prevent pattern defects in the early embryo (43, 139–141, 185). This translational repression is mediated by a 184-nt translational control element (TCE) in the *nanos* 3′-UTR (140) that contains two separable Smaug recognition elements (SREs), which bind a translational repressor to be described in the next section (141). These elements map within evolutionarily conserved regions of the *nanos* 3′-UTR (185). SRE1 lies between nucleotides (nt) 25 and 40 (141) within a 90-nt region (nt 1–90 of the 3′-UTR), which was shown independently to confer translational repression (185). SRE2 maps to nt 130–144, downstream in the 3′-UTR (141), within an adjacent 88-nt region (nt 91–178), which independent analyses showed has limited ability to repress translation (185). The SREs can form stem-loops (14–23 nt in length) with a highly conserved loop sequence (CUGGC) while the stem sequence is not conserved. Point mutations in the loops abolish binding of Smaug protein and eliminate translational repression of *nanos* RNA.

The BREs in the *oskar* 3′-UTR and the SREs in the *nanos* 3′-UTR are clear examples of discrete elements that are repeated within the 3′-UTRs and are largely functionally redundant.

A translational control element has also been mapped within the *Cyclin B* 3′-UTR, to a 39-nt region distinct from the posterior localization element (see above) (60, 182). In this case, the translational control element represses translation of localized maternal *Cyclin B* mRNA until late stage 14, about 11 h after fertilization. Deletion of the element results in premature translation, starting an hour after fertilization (182). The functional significance of this translational control has not been determined.

Primary, Secondary, Tertiary, and Quaternary Structures

A final question to be addressed here is the nature of the localization and translational control elements themselves. That is, is it the primary, secondary, tertiary, or quaternary structure that is recognized by the localization and translational control machinery? Instances of each of these possibilities have been mentioned in the preceding discussion. Several conserved primary sequence elements have been defined. For example, the SREs in the *nanos* 3′-UTR include a highly conserved loop sequence (CUGGC) recognized by Smaug (141), and the BREs in the *oskar* 3′-UTR contain a highly conserved sequence

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

[U(G/A)U(A/G)U(G/A)U] important for Bruno binding (184, 186). Similarly, a conserved sequence in *Vg1* and several other 3′-UTRs (*TGFβ-5*, *Xwnt-11*, *Xlsirt*, *tau*, *oskar*, *nanos*, and *gurken*) has been implicated in localization (187). As mentioned above, the chicken *β-actin* 3′-UTR contains two regions that confer peripheral localization (171); these regions each contain two conserved motifs (GGACT and AATGC).

The importance of RNA secondary structure in localization has been clear for some time (e.g. *bicoid* 3′-UTR) (166, 167). Conserved primary sequence elements are often parts of a stem-loop (e.g. SREs) and are likely to be bound by factors that interact with single-stranded RNA. Other examples of important stem-loop structures are the *K10* TLE (181) and, possibly, the *Add-hts* ALE1 (KL Whittaker, D Ding, WW Fisher, HD Lipshitz, manuscript in preparation).

Initially it was proposed that tertiary structure is also important for localization based on the discovery of complementarity between two loops within stem-loop III of the *bicoid* 3′-UTR (166, 167). These complementary regions actually undergo quaternary interactions (i.e. between different 3′-UTR molecules) mediated by the double-stranded RNA-binding Staufen protein (see below) (163, 164).

TRANS-ACTING FACTORS INVOLVED IN RNA LOCALIZATION AND TRANSLATIONAL CONTROL OF LOCALIZED RNAs

The previous section outlined the *cis*-acting sequences that function in RNA localization. It also discussed the sequences involved in localization as well as the elements involved in translational control of mRNAs during or after localization. Here, the focus is on *trans*-acting factors that function during RNA localization. These factors can function in RNA localization per se or in translational control during or after localization. The latter class of factors is included only if translational control is related directly either to the localization process or to the functional significance of RNA localization.

Identification of Trans-Acting Factors

Three strategies have led to the identification of *trans*-acting factors that function in RNA localization and/or translational control of localized RNAs. The first strategy, genetic definition of genes involved in RNA localization followed by molecular cloning of the genes and molecular biological and biochemical analyses of their encoded products, is restricted to *D. melanogaster* and *S. cerevisiae*. Examples are *Drosophila* Bicaudal-C, Bicaudal-D, Exuperantia, Homeless, K10, Squid, Staufen, Swallow, and Vasa. Although this strategy ensures that the gene product is involved in RNA localization, it cannot be

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

determined at the outset whether the effects on RNA localization are direct or indirect.

A second strategy proceeds in the opposite direction by starting with biochemical searches for factors that bind defined RNA elements involved in localization and/or translational control. Ultimately, the gene encoding the identified factor is cloned and, in *Drosophila*, also mutated in order to assay function of the endogenous protein. With this method, it is known from the outset that the factor interacts directly with the target RNA; however, one has no assurance that the identified protein will indeed function in RNA localization/translational control rather than in some other aspect of RNA metabolism. Examples of *trans*-acting factors identified in this way are Bruno, Smaug, and Exl in *Drosophila*, and Vg1 RBP and Vera in *Xenopus*.

A final approach (not so much a strategy) that has led to the identification of factors involved in RNA localization has derived from molecular screens for gene products (RNA or protein) with interesting intracellular distributions (e.g. localization) or with interesting molecular homologies (e.g. RNA-binding motifs). In this case, as for the second strategy described above, one has no prior indication that the gene product functions in localization or translational control. However, if it is present in the right place at the right time, and possesses the appropriate molecular properties, the gene product may have a function in the process of interest. Examples of gene products identified in this way are Oo18 RNA-binding protein (Orb) and *Pgc* RNA in *Drosophila*, the *Xlsirt* RNAs in *Xenopus*, and the *YC* RNA in ascidians.

Factors That Interact Directly with Defined RNA Elements

STAUFEN PROTEIN (*DROSOPHILA*) Alleles of the *staufen* gene were first recovered as maternal effect mutations with defects in anterior and posterior (abdominal) pattern in the *Drosophila* embryo (188). It was implicated in *bicoid* RNA localization to the anterior since *bicoid* RNA is partially delocalized in early embryos produced by mutant mothers (7, 32, 189). In *staufen* mutants *oskar* RNA is maintained at the anterior of the oocyte until stage 10 when it delocalizes (25) (in wild-type oocytes *oskar* RNA is transported from the anterior pole to the posterior by stage 9). Therefore, *staufen* is essential for initiation of posterior transport of *oskar* RNA. Moreover, weak *staufen* alleles show normal posterior localization of *oskar* RNA at stage 9, but the posterior localization is lost in later oocytes (stage 11) indicating that Staufen is also required to maintain posteriorly localized *oskar* RNA (190). During oogenesis Staufen protein first appears uniformly in stage 3–4 egg chambers, and by stage 8 it is present in a ring at the oocyte anterior as well as at the posterior of the oocyte (179). By stage 10B Staufen protein is at the posterior pole of oocyte; therefore, *oskar*

RNA and Staufen protein colocalize. In the early embryo, Staufen protein colocalizes with *oskar* RNA at the posterior pole and with *bicoid* RNA at the anterior pole (179).

Staufen is a double-stranded RNA-binding protein (178). Varying the amount of the Staufen target RNAs (*bicoid* or *oskar*), or of Staufen protein, indicates that the amount of Staufen protein recruited to the anterior or posterior pole depends on the amount of *bicoid* or *oskar* RNA present; thus the RNA targets rather than Staufen protein are limiting. Injected in vitro transcribed *bicoid* 3′-UTR RNA recruits Staufen protein into particles that colocalize with microtubules near the site of injection in the early embryo (163). The formation of these particles requires specific 3′-UTR elements previously defined as important for *bicoid* RNA localization (see above). Specifically, evidence suggests that two single-stranded regions of stem-loop III within the *bicoid* 3′-UTR form intermolecular double-stranded RNA hybrids (i.e. via quaternary interactions) that are bound by Staufen protein (164). Staufen protein also interacts later in embryogenesis with the *prospero* 3′-UTR in neuroblasts and is necessary for basal localization of *prospero* transcripts (127).

Given that Staufen interacts with the best-characterized 3′-UTRs of localized RNAs (those in *bicoid* and *oskar* RNAs), that it is an RNA-binding protein, and that mutations exist both in the *staufen* gene and in the genes that encode its target RNAs, Staufen is by far the best-understood *trans*-acting factor involved in RNA localization.

EXL PROTEIN (*DROSOPHILA*) BLE1 is a 53-nt element in the *bicoid* 3′-UTR that is sufficient (when present in two copies but not one) to direct early nurse cell–oocyte transport and anterior localization of RNA (see above) (176). 2xBLE1 was used in UV-crosslinking assay to search for directly interacting proteins (177). A single protein of 115 kDa (Exl) was found to bind 2xBLE1 but not 1xBLE1, consistent with a role in localization. Definition of Exl-binding sites within BLE1 and mutation of these sites gave results consistent with a role for Exl in BLE1-mediated anterior localization. Exl might interact directly with BLE1 in the *bicoid* 3′-UTR during localization, and it might mediate Exuperantia protein interaction with *bicoid* RNA in the localization particles (177) (see below for discussion of Exuperantia). To date, the gene encoding Exl has not been cloned, and mutations in the gene have not been identified.

BRUNO PROTEIN (*DROSOPHILA*) Bruno, an 80-kDa protein, was identified in a UV-crosslinking screen for *trans*-acting factors that bind the *oskar* 3′-UTR (184). There are three binding sites (BREs) in the *oskar* 3′-UTR that share a 7- to 9-nt motif. Mutations in these elements abolish Bruno binding in vitro

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

(184). Endogenous *oskar* mRNA is translated only after the transcript is localized to the posterior of the oocyte in stages 8–9 (184). An *oskar* [BRE$^-$] transgene results in premature translation of *oskar* RNA during stages 7–8, prior to *oskar* RNA localization, producing gain-of-function phenotypes (double-posterior or posteriorized embryos) (184), consistent with spatially inappropriate Oskar protein expression (75, 76). BRE elements can confer translational repression on a heterologous transcript (184). Bruno-mediated translational control of other transcripts is suggested by the ability of Bruno protein to bind *gurken* RNA (184).

The gene encoding Bruno was recently cloned (186). The sequence reveals RNP/RRM-type ribonucleoprotein RNA-binding domains consistent with direct interaction with RNA. The RNP/RRM-domain RNA-binding motif was first defined in yeast mRNA poly(A)-binding protein and mammalian hnRNP protein A1 (reviewed in 191). The gene encoding Bruno is a previously identified genetic locus, *arrest*, which is necessary for female as well as male fertility (192, 193). This observation, together with the fact that *arrest* mutants display defects in oogenesis prior to the time that Bruno binds *oskar* RNA, suggests that Bruno regulates other transcripts in addition to *oskar* RNA.

Bruno also interacts with Vasa protein as assayed by far-Western analysis (186). Vasa is itself an RNA helicase related to eIF-4A, which functions in translation initiation (194–196). This suggests a possible mechanism for Bruno; Bruno may protect *oskar* RNA from premature translational activation by the Vasa protein (186).

Identification and cloning of Bruno, and its correlation with a previously identified genetic locus, is the first instance in which a *trans*-acting factor involved in translational control of a localized RNA has been identified biochemically and then studied both molecularly and genetically.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

SMAUG PROTEIN (*DROSOPHILA*) As described above, translational repression of unlocalized *nanos* mRNA (43, 140, 141) is accomplished through two elements that bind in vitro to Smaug, a 135-kDa protein (141). Mutation of these SREs abolishes Smaug binding in vitro. Embryos from *nanos* [SRE$^-$] transgenic mothers lose head structures, which is consistent with translation of *nanos* RNA throughout the embryo rather than solely at the posterior (141). Smaug binding alone is sufficient to mediate translational repression since a transgene containing three SREs but no other part of the *nanos* 3′-UTR is translationally repressed (141). The gene encoding Smaug has not been cloned.

VG1 RNA-BINDING PROTEIN (RBP) (*XENOPUS*) UV crosslinking has also been used to identify *trans*-acting factors that bind to the *Vg1* 3′-UTR, which functions in vegetal localization of *Vg1* RNA in *Xenopus* oocytes (197). The crosslinking experiments led to the identification of a 69-kDa protein called Vg1

RNA-binding protein (RBP). Vg1 RBP may also bind in vitro to the 3′-UTR of a second vegetal pole–localized RNA, *TGFβ-5* (187, 197), but not to *An2* RNA, which is localized to the animal hemisphere. The precise role of Vg1 RBP in vegetal RNA localization is not clear. Evidence indicates that it mediates the association of *Vg1* RNA with microtubules (199), which are necessary for vegetal RNA localization (see above). Based on the results of UV-crosslinking experiments Vg1 RBP is likely to be a member of an RNP complex containing up to six proteins plus *Vg1* mRNA (200). Six protein bands (one of which is likely to be Vg1 RBP) were identified from stage II–III oocyte extracts, corresponding to the period during which *Vg1* RNA is localized (200). Fewer proteins were labeled in earlier and later stage oocyte extracts, at times during which *Vg1* RNA is not being localized.

VERA PROTEIN (*XENOPUS*) In a search for *trans*-acting factors that bind the *Vg1* localization element a new 75-kDa protein, Vera, was purified (154). A mutant form of the *Vg1* localization element (deleted for three out of four repeated sequence motifs) that does not bind Vera in vitro exhibits impaired localization in vivo (154). Vera protein co-sediments with Trap-α, an integral membrane protein associated with the protein translocation machinery of the endoplasmic reticulum (ER) (154). Vera may link *Vg1* mRNA to the vegetal ER subcompartment while the ER (along with *Vg1* RNA) is transported via microtubules to the vegetal pole.

Other Factors That Function in RNA Localization

EXUPERANTIA PROTEIN (*DROSOPHILA*) The genetic analyses that led to the identification of *staufen* as functioning in *bicoid* RNA localization also led to the identification of *exuperantia* (188, 189). The first observable *bicoid* RNA localization defect in *exuperantia* mutants is loss of apical transcript localization in nurse cells (32) at a stage at which Exuperantia protein is localized around the nurse cell nuclei (201, 202). Exuperantia protein is highly concentrated in the anterior cortex of the oocyte between stages 8 and 10 (162, 201). In stage 10 egg chambers mutant for *exuperantia*, *bicoid* RNA delocalizes from the anterior of the oocyte (7, 150). In late oocytes (stage 14) mutant for *exuperantia*, *bicoid* RNA is released from the microtubule-based cytoskeleton (147). However, Exuperantia protein is not present in late oocytes or in embryos, indicating that Exuperantia is involved in establishing but not in maintaining anterior *bicoid* RNA localization (201, 202).

Visualization of an Exuperantia-GFP fusion protein in live oocytes (162) demonstrates that Exuperantia is present in large particles that are transported from the nurse cells into the oocyte through the ring canals (see above). Transport of these Exuperantia-containing particles appears to be a multistep process: Colchicine-sensitive steps are transport within the nurse cells and

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

anchoring at the anterior of the oocyte; whereas transport through the ring canals into the oocyte is insensitive to both microtubule and microfilament inhibitors (162; WE Theurkauf, TI Hazelrigg, personal communication). Exuperantia may function in this microtubule-independent transport of *bicoid* transcripts through the ring canals into the oocyte (151).

Taxol stabilizes microtubules and causes aberrant microtubule bundles in the oocyte that contain ectopically localized *bicoid* transcripts (150). Taxol-treated *exuperantia* mutant oocytes do not exhibit ectopic *bicoid* transcript localization (150). The microtubule networks in *exuperantia* oocytes are normal. These results are consistent with *bicoid* RNA association with ectopic microtubules requiring Exuperantia protein.

Deletion of the *cis*-acting element BLE1 from the *bicoid* 3′-UTR mimics the *bicoid* transcript delocalization defects caused by *exuperantia* mutants (176). However, BLE1 specifically binds a protein called Exl (see above) and Exuperantia alone can bind RNA only nonspecifically (177). Exuperantia protein may interact with Exl protein in *bicoid* RNA localization particles. While Exuperantia does not interact specifically with *bicoid* RNA, it does function specifically in *bicoid* RNA localization; other anteriorly localized RNAs such as *Add-hts*, *Bicaudal-D*, *K10*, and *orb* are not delocalized in *exuperantia* mutant oocytes (16, 37).

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

SWALLOW PROTEIN (*DROSOPHILA*) The third genetic locus initially shown to be necessary for *bicoid* transcript localization during oogenesis is *swallow* (7, 189). Subsequent analyses implicated it in the cortical and anterior localization of a second RNA, *Add-hts* (16, 174; KL Whittaker, D Ding, WW Fisher, HD Lipshitz, manuscript in preparation). The *swallow* locus is not necessary for the transient anterior positioning of other RNAs such as *Bicaudal-D*, *K10*, and *orb* (37). Swallow protein maintains cortical localization of transcripts such as *bicoid* and *Add-hts* as well as of RNPs such as the polar granules (44, 45). With regard to *bicoid*, Swallow appears to be involved in maintaining rather than establishing *bicoid* transcript association with microtubules since taxol-treated *swallow* oocytes exhibit ectopic *bicoid* transcript localization (150). The Swallow protein may possess a highly divergent RNP/RRM motif suggestive of a direct interaction with RNA (203). Swallow protein is distributed throughout the oocyte at stages 5–7 and at its anterior cortex at stages 8–10, consistent with localization of *Add-hts* and *bicoid* RNAs to these regions (44, 45, 204).

OSKAR PROTEIN (*DROSOPHILA*) Nonsense mutants such as *oskar*^{54} show abnormal *oskar* RNA localization (24, 25). In these mutants *oskar* RNA localizes to the posterior in stage 8 but subsequently delocalizes, indicating a role for Oskar protein in anchoring *oskar* RNA at the posterior. Directly or indirectly,

Oskar protein's role in *oskar* transcript localization is mediated by the *oskar* 3′-UTR since a chimeric *oskar* 3′-UTR construct localizes to the posterior and is maintained there in wild type but fails to be maintained there in an *oskar*^{54} mutant (190). Further support for this scenario is the observation that in *D. melanogaster*, a transgenic *D. virilis oskar* RNA is able to localize to the posterior but fails to maintain its position at the oocyte posterior in the absence of *D. melanogaster* Oskar protein (205). *D. virilis* Oskar can direct posterior abdominal patterning but not pole-cell formation. This observation indicates that pole-cell formation may require a high concentration of Oskar protein and that this is provided for by anchoring high concentrations of *oskar* RNA at the posterior. Oskar protein nucleates the formation of the posterior polar granules, organelles that function in germ-cell formation and specification (see below). Oskar protein may interact directly with the *oskar* RNA's 3′-UTR to maintain transcript localization. Alternatively, Oskar function may be indirect in nucleating formation of polar granules that in turn are needed for *oskar* RNA localization.

VASA PROTEIN (*DROSOPHILA*) Vasa protein is a component of the perinuclear "nuage" material in the nurse cells and also of the polar granules at the posterior pole of the *Drosophila* oocyte (194, 196, 206–208). Loss of *vasa* function results in destabilization of the polar granules and delocalization of posteriorly localized RNAs (188, 194, 196, 206–208). Vasa protein is a founding member of the DEAD-box family of ATP-dependent RNA helicases (194, 207), and it binds duplex RNA (196). There is no evidence for specific binding of Vasa to any localized RNA; however, far-Western analysis has demonstrated that Vasa protein interacts directly with Bruno protein, which in turn binds directly to the BRE translational control elements in the *oskar* RNA's 3′-UTR (see above) (186). Transcripts encoding the zebrafish homolog of Vasa are localized subcellularly beginning at the two-cell stage and segregate into the primordial germ cells (209). This observation suggests likely evolutionary conservation of Vasa function in metazoan germ plasm.

HOMELESS PROTEIN (*DROSOPHILA*) Homeless is another member of the DEAD/DE-H family of RNA-binding proteins (40). Its amino terminal portion contains a region that bears homology to yeast splicing factors PRP2 and PRP16 and to the *Drosophila* Maleless protein (40). Transport and localization of *gurken*, *oskar*, and *bicoid* transcripts are severely disrupted in *homeless* mutant ovaries, which also contain reduced amounts of *K10* and *orb* transcripts (40). In contrast, *Bicaudal-D* and *Add-hts* transcript localization is unaffected (40). It is unknown whether Homeless protein interacts directly with any of the affected transcripts.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

ORB PROTEIN (*DROSOPHILA*) Orb protein contains two RNP/RRM-type RNA-binding domains and functions in antero-posterior and dorso-ventral patterning during *Drosophila* oogenesis (23, 149, 210). *orb* mutants affect the transport and localization of several RNAs including *Add-hts*, *Bicaudal-D*, *K10*, *gurken* and *oskar* (149, 210). For example, in wild-type oocytes during stages 8–10, *gurken* mRNA is usually restricted to the dorsal side of the nucleus at the antero-dorsal pole. In *orb* mutants, however, *gurken* transcripts are present throughout the entire anterior of the oocyte. In the wild type, *oskar* mRNA is localized to the posterior pole of the oocyte. In *orb* mutants *oskar* transcripts are distributed throughout the oocyte. Direct binding of Orb protein to either RNA has not been demonstrated.

SQUID PROTEIN (*DROSOPHILA*) Squid is a member of the hnRNP family of RNA-binding proteins and was identified as functioning in dorso-ventral axis formation (211). There are three protein isoforms that share a common amino terminus containing two RNA-binding motifs. Two of these isoforms, Squid-A and Squid-B, are present in the oocyte. Squid is required for the correct dorso-anterior localization of *gurken* mRNA in the oocyte. In *squid* mutant ovaries, *gurken* transcripts are localized throughout the anterior of the oocyte rather than just antero-dorsally (211). Since *gurken* is unusual in that it is transcribed in the oocyte nucleus (R Cohen, personal communication), Squid's function in *gurken* transcript localization may initiate in the oocyte nucleus. It is unknown whether Squid interacts directly with the *gurken* mRNA or with the *gurken* pre-mRNA. As for K10 (below), Squid may function in vectorial transport of *gurken* transcripts out of the oocyte nucleus.

K10 PROTEIN (*DROSOPHILA*) *K10* gene function is required during stage 8 of oogenesis for localization of *gurken* mRNA adjacent to the oocyte nucleus at the antero-dorsal tip of the oocyte (21, 212). K10 protein is restricted to the oocyte nucleus (213) but does not regulate *gurken* transcript production or stability (212). Since *gurken* is transcribed in the oocyte nucleus (R Cohen, personal communication) and becomes restricted to the oocyte cytoplasm dorso-anteriorly to the oocyte nucleus, K10 protein might function specifically in vectorial nucleo-cytoplasmic transport of *gurken* transcripts (R Cohen, personal communication). Whether this function is through direct interaction with the *gurken* mRNA is not known at present.

BICAUDAL-C PROTEIN (*DROSOPHILA*) Mutations that reduce *Bicaudal-C* gene dosage result in defects in RNA localization in the oocyte: Most *oskar* RNA remains at the anterior pole of the oocyte and early embryo instead of being transported to the posterior by stage 9 (18). Possibly as a consequence, in these *Bicaudal-C* mutants, *nanos* RNA is localized ectopically near the anterior pole

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

in patches on the dorsal and ventral sides rather than at the posterior pole as in wild type (18). These embryos develop a bicaudal (double-abdomen) phenotype. *Bicaudal-C* mutations have no effect on *gurken* or *orb* transcript localization to the anterior pole. Females homozygous for strong *Bicaudal-C* alleles produce oocytes that do not form anterior chorion as a consequence of defects in follicle cell migration over the oocyte anterior. Bicaudal-C is a transmembrane protein that has two conserved cytoplasmic domains (18): an Eph domain that is present in transmembrane receptor tyrosine kinases and is involved in signal transduction, and a KH domain that has been implicated in binding of single-stranded DNA or RNA. Since *Bicaudal-C* RNA is localized to the anterior of the oocyte, it is reasonable to assume that the Eph domain functions in the intercellular signaling from oocyte to follicle cells that programs their migration (18). The KH domain might bind to and interact with the *oskar* transcripts during their localization, possibly functioning in their transfer to the machinery that transports RNA to the posterior (see above) (18).

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

BICAUDAL-D PROTEIN (*DROSOPHILA*) Bicaudal-D function has been covered with respect to RNA transport and the oocyte cytoskeleton above. The Bicaudal-D protein includes a region with homology to the coiled-coil domains of several cytoskeletal proteins and is required for maintenance of *oskar* RNA localization at the posterior pole of the oocyte (160).

EGALITARIAN PROTEIN (*DROSOPHILA*) In *egalitarian* mutants the *Bicaudal-D*, *orb*, and *K10* RNAs do not accumulate in the oocyte (25). In addition, these RNAs no longer coprecipiate with the cytoskeletal fraction of oocytes as in the wild type (147). Egalitarian protein contains regions homologous to c10G6.1 from *Caenorhabditis elegans*, an EST from *Arabidopsis thaliana*, and ribonuclease D from *Haemophilus influenzae* (20). It is also predicted to include a coiled-coil region. Egalitarian protein localization to and within the oocyte is dependent on microtubules (20). Further, the Egalitarian and Bicaudal-D proteins copurify (20). Egalitarian and Bicaudal-D may be components of the cytoskeletal apparatus involved in RNA localization.

BULLWINKLE (*DROSOPHILA*) Mutations in the *bullwinkle* gene have several defects in posterior body patterning (214, 215). The *bullwinkle* gene is required to localize *oskar* transcripts to the posterior of the oocyte, to maintain *oskar* RNA at posterior, and to regulate the level of *oskar* protein (214, 215). Cloning of the *bullwinkle* gene has not been reported.

AUBERGINE (*DROSOPHILA*) While previously *aubergine* had been implicated in dorso-ventral body patterning (192), two new alleles were identified in a recent genetic screen for genes involved in posterior body patterning (214, 216).

Aubergine functions to enhance the translation of *oskar* mRNA in the ovary, mediated through the *oskar* 3′-UTR (216). *Aubergine*'s enhancement of *oskar* translation is independent of Bruno-mediated repression of translation since an *oskar* [BRE$^-$] transgenic RNA still requires *aubergine* function for its translation (216). Cloning and molecular analysis of *aubergine* have not been reported.

XLSIRT RNA (*XENOPUS*) *Xlsirt*s are a family of nontranslatable, interspersed repeat transcripts that localize to the vegetal cortex of *Xenopus* oocytes (see above) (97, 161). These RNAs are involved in the localization of *Vg1* but not *Xcat-2* transcripts. It is unknown whether *Xlsirt* and *Vg1* RNAs interact directly or whether *Xlsirt* RNA function in *Vg1* transcript localization is indirect via the cytoskeletal network or as part of a localization particle/organelle.

PGC RNA (*DROSOPHILA*) In *Drosophila* a non-protein-coding RNA, *Pgc*, is a component of the posterior polar granules and is required for pole cells to migrate normally and to populate the gonad (26). Reduction of *Pgc* RNA at the posterior pole results in a reduction in the amount of posteriorly localized *nanos* and *germ cell-less* RNA and Vasa protein (26). It is unknown whether *Pgc* RNA interacts with other posteriorly localized RNAs and/or with protein components of the polar granules.

YC RNA (*STYELA*) As described above, the noncoding *YC* RNA is localized in the yellow crescent of Ascidian eggs and embryos (101). The 3′-UTR of *PCNA* RNA contains a 521-nt region of complementarity to *YC* RNA (100), while the 5′-UTR of *ribosomal protein L5* mRNA exhibits 789 nt of complementarity to *YC* RNA (102). *YC* RNA may interact directly with these two RNAs in vivo (100–102); however, no such interaction has been demonstrated, and its function remains unclear (see above).

Summary

In summary, genetic and molecular strategies have identified a host of *trans*-acting factors that function in transcript localization. To date, although many of these are homologous to known RNA-binding proteins, only a handful have been shown to interact directly with specific localized RNAs. Many localized RNAs have collections of discrete *cis*-acting localization elements that mediate distinct aspects of their localization. It might therefore be predicted that many different *trans*-acting factors will function during localization of any one RNA, each binding to a different type of element and each possibly functioning at a different time and intracellular location. RNA localization particles are likely to consist of these directly interacting factors in addition to numerous others that are involved in linking the RNA to the cytoplasmic translocation machinery

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

as well as anchoring it at its intracellular target site. It would be surprising if evolution has not also utilized the base-pairing capability of RNA in the translocation and anchoring process.

DEVELOPMENTAL FUNCTIONS OF RNA LOCALIZATION

The cellular functions of RNA localization have been reviewed extensively (see e.g. 1, 217) and so are considered here only briefly. First, for mRNAs, localization directs high-level synthesis of the encoded protein at the site of localization. Thus if protein function requires a high concentration (e.g. *Drosophila* Oskar in the polar granules), this requirement can be met through mRNA localization. Second, localizing an mRNA also excludes protein synthesis from other parts of the cell, thus reducing the amount of protein present in those regions. Often, not only is local protein synthesis directed by mRNA localization, but translational control mechanisms actually prevent translation of unlocalized RNA either during RNA transit to the target site (e.g. *Drosophila oskar* RNA) or of unlocalized RNA that remains after transcript localization is complete (e.g. *Drosophila nanos* RNA).

A third postulated function of mRNA localization is to direct specific protein isoforms to particular regions of the cell. Often this is accomplished by alternative pre-mRNA splicing such that different 3′-UTRs direct different isoforms to different cytoplasmic domains (e.g. *Drosophila Add-hts* RNA). Fourth, intracellular localization is used as a mechanism to segregate RNAs unequally between the products of cell division, particularly when these divisions are asymmetric (e.g. *ASH1* transcripts during yeast budding, *prospero* transcripts during *Drosophila* neuroblast division). Fifth, certain non-protein-coding RNAs are localized (e.g. *Xenopus Xlsirt*s or *Drosophila Pgc* RNAs). The detailed role of these RNAs and of their localization is currently under intensive study. These RNAs may serve as structural components of localization particles or organelles such as the germinal granules. Alternatively, they may function in the RNA localization or anchoring process, possibly through sequence complementarity to mRNAs that are being localized.

Specification of the Anterior-Posterior and Dorsal-Ventral Axes of the Drosophila Oocyte

As mentioned above, the *gurken* mRNA is unusual in that it is synthesized in the oocyte nucleus (R Cohen, personal communication). It is localized to the posterior pole of the oocyte at stage 7, then to both the anterior and posterior poles at stage 8, and finally to the antero-dorsal pole from late stage 8 through stage 10 (21, 218). Gurken protein is a TGFα-like secreted growth

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

factor (21). Establishment of the antero-posterior and dorso-ventral axes of the oocyte is accomplished between stages 7 and 9 of oogenesis through signaling between the oocyte and the surrounding follicle cells (34, 35). The Gurken protein functions as a key signal from the oocyte to the follicle cells in both of these processes. First, due to posterior-localized *gurken* RNA, local production of Gurken protein at the posterior of the oocyte signals to the posterior follicle cells. This signaling is essential for establishing the antero-posterior oocyte axis and the polarization of the oocyte microtubule-based cytoskeleton that plays a crucial role in RNA localization. Subsequently, antero-dorsal localized *gurken* RNA directs local Gurken protein synthesis, enabling oocyte–nurse cell signaling that establishes the dorso-ventral axis of the egg chamber. The dorso-anterior localization of *gurken* mRNA depends on anterior migration of the oocyte nucleus on the polarized microtubule cytoskeleton. Thus the antero-posterior axis is primary and the establishment of the dorso-ventral axis secondary (35). Both axes depend on localization of *gurken* mRNA for localized signaling. If *gurken* mRNA is mislocalized or delocalized, for example, by mutating the *K10*, *squid*, or *orb* genes, severe defects in the formation of both axes result.

Specification of Anterior Cell Fates in the Drosophila Embryo

Shortly after fertilization, *bicoid* mRNA is translated (219, 220). Since the embryo is syncytial, Bicoid protein diffuses away from its site of translation at the anterior pole, forming an antero-posterior protein gradient with its peak at the anterior tip (219, 220). Since Bicoid is a homeodomain-containing transcription factor, its function is to activate zygotic transcription of pattern-specifying genes in the syncytial nuclei in the anterior half of the embryo. It does this in a concentration-dependent fashion (221–223). For example, the *hunchback* gene contains high-affinity Bicoid-binding sites in its transcriptional control region, so its transcription is activated by low as well as high Bicoid concentrations throughout the anterior half of the embryo. In contrast, genes such as *orthodenticle* and *empty spiracles* have lower affinity Bicoid-binding sites and so are activated only by higher Bicoid protein concentrations in the more anterior part of the embryo. In this way, different combinations of zygotic pattern genes are activated in different subsets of the anterior part of the embryo leading to different cell fates within this region (e.g. head more anteriorly, thorax more posteriorly). *Bicoid* mRNA localization controls the amount of Bicoid transcription factor in different regions and thus specifies distinct cell fates. Delocalization of *bicoid* mRNA can be accomplished by mutating genes that encode *trans*-acting factors that function in *bicoid* transcript localization (7, 32, 189) (e.g. *exuperantia*, *swallow*, *staufen*). Delocalization results in lower

levels of Bicoid protein at the anterior pole than in the wild type. As a consequence, acronal and head structures cannot be specified.

Localization of *bicoid* mRNA serves two additional functions. First, mislocalization of *bicoid* RNA to the posterior pole can result in developmental defects in the posterior part of the embryo through cells mistakenly adopting anterior fates (224). Thus a corollary function of anterior *bicoid* transcript localization is to prevent Bicoid protein synthesis in the posterior of the embryo. Translational control mechanisms also prevent Bicoid protein synthesis at the posterior (141, 205, 225). Second, the Bicoid homeodomain protein can function not only as a transcription factor but also can directly bind RNA and translationally repress mRNAs such as *caudal* through interaction with the *caudal* 3′-UTR (226, 227). Thus anterior localization of *bicoid* mRNA and the resultant Bicoid protein gradient creates a reverse gradient of Caudal protein with its peak at the posterior pole. Caudal protein is involved in specifying pattern in the posterior of the early embryo (228, 229).

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Specification of Abdominal Cell Fates in the Drosophila Embryo

A key player in abdominal cell fate specification is Nanos, a Zinc-finger-containing protein (56, 230). The *nanos* mRNA is localized at the posterior pole of the late oocyte and early embryo, although some unlocalized RNA is present throughout the embryo (56, 231). After fertilization, the posteriorly localized *nanos* RNA in the syncytial embryo is translated (there is repression of unlocalized *nanos* RNA translation by Smaug protein). This translation leads to a gradient of Nanos protein with a peak at the posterior pole (139–141, 185, 231). Unlike *bicoid*, which controls anterior cell fates by a combination of transcriptional control of target genes and direct translational repression of *caudal* mRNA in the anterior, all of the Nanos protein's effects in abdominal patterning derive from its translational repression of target RNAs. One target, *hunchback* maternal RNA, is distributed uniformly in the early embryo (232, 233) and encodes a Zinc-finger transcription factor that specifies anterior cell fates (234–236). Thus, if Hunchback protein were synthesized in the posterior of the embryo, posterior cells would mistakenly adopt anterior fates. Nanos protein in the posterior of the embryo prevents this by translationally repressing *hunchback* RNA. The Pumilio protein, previously shown to be important for abdominal patterning (237), specifically binds to Nanos response elements (NREs) in the *hunchback* 3′-UTR, recruiting Nanos through protein-protein interactions (140). If *nanos* RNA is misexpressed throughout the embryo by mutation of its SREs, head defects result, probably through repression of *bicoid* translation by Nanos protein since the *bicoid* 3′-UTR also contains NREs (141). Misexpression of high levels of Nanos protein in the anterior results in bicaudal

embryos (231). Thus the combination of posterior localization of *nanos* mRNA and the translational repression of unlocalized *nanos* transcripts plays a crucial role in patterning the abdomen of *Drosophila*.

Assembly of Polar Granules and Specification of Germ Cells in the Drosophila Embryo

oskar mRNA is localized to the posterior pole of the stage 9 oocyte. Translation of *oskar* RNA at this site nucleates the formation of the posterior polar granules and polar plasm (25, 75, 76). The polar granules and posterior polar plasm serve two functions. The first is the anchoring of *nanos* transcripts at the posterior (75, 76). Disruption of the polar granules results in delocalization of *nanos* RNA, translational repression by Smaug, and ultimately the production of embryos without abdomens. The second role of polar granules is to specify the formation of germ (pole) cells and to restrict their formation to the posterior tip of the embryo (25, 75, 76). Disruption of *oskar* function results in an inability to nucleate polar granules and, consequently, absence of pole cells (238). Alternatively, misexpression of Oskar protein throughout the oocyte during *oskar* RNA localization (184, 186), overexpression of *oskar* RNA throughout the oocyte (75), or mislocalization of *oskar* RNA and protein to the anterior pole of the oocyte (76) all result in severe pattern defects. In the latter two situations ectopic pole cells form at or near the anterior of the embryo. The mechanisms by which the polar granules specify the formation and function of the pole cells in the early embryo are not yet fully understood but appear to require the function of several other posteriorly localized RNAs such as *Pgc*, *mtlrRNA*, *nanos*, and *germ cell-less* (26, 57, 133, 134, 136, 137, 239, 240). Thus, both the establishment of the polar granules, and their function in pole cell formation, require RNA localization.

Signaling of Dorso-Ventral Axis and Mesoderm Induction in the Xenopus Embryo

The animal-vegetal axis of the *Xenopus* embryo is established during oogenesis. The three germ layers of the early embryo (ectoderm, mesoderm, and endoderm) are established along the animal-vegetal axis (72, 73). The darkly pigmented animal hemisphere of the oocyte gives rise to ectoderm while the vegetal hemisphere cells become endoderm. The mesoderm is derived from animal hemisphere cells that lie adjacent to the vegetally derived mesoderm. Mesodermal development is not autonomous but is a result of inductive interactions from the endoderm (72, 73).

Asymmetrically distributed RNAs localized to the vegetal hemisphere that encode secreted growth factors such as Vg1 and TGFβ-5 have been implicated in mesoderm induction (5, 241–243). The secreted growth factor TGF-β1 can

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

act synergistically with another growth factor, bFGF, to induce mesoderm, whereas antibodies against TGF-β2 can reduce mesoderm induction when injected into *Xenopus* embryos. Since *Vg1* and *TGF-β5* RNAs are localized to the vegetal hemisphere from which the inducing signal derives, and since Vg1 and TGF-β5 proteins are related, respectively, to TGF-β1 and 2, these proteins are strong candidates for mesoderm-inducing signals (5, 241–243). Indeed, engineered processed Vg1 protein (in the form of BMP2/4-Vg1 fusion protein) can function as a mesoderm inducer when ectopically expressed in embryos (241, 242).

The orientation of the dorso-ventral axis of the early embryo is not established prior to fertilization. Rather, the sperm entry point in the animal hemisphere establishes this axis in part by causing an oriented cytoplasmic rearrangement (72, 73). This rearrangement relocates interior cytoplasm (endoplasm) relative to the stationary cortical cytoplasm. Treatments, such as UV-irradiation, prevent cytoplasmic rearrangement and ventralize the embryo (i.e. prevent formation of the dorsal-most tissues). Maternal *Xwnt-11* mRNA is localized vegetally in oocytes and early embryos (98). Injection of *Xwnt-11* RNA into embryos that have been ventralized by UV-irradiation substantially rescues the UV-induced defect by inducing formation of dorsal tissues such as somitic muscle and neural tube (98). This observation suggests that Xwnt-11 protein functions during normal embryogenesis in dorso-ventral axis formation and that localization of *Xwnt-11* mRNA and protein may play a role in induction of this axis.

The inductive events discussed previously are complex both at the level of inducing signals and at the level of mesodermal cell fate outcomes. The inability to genetically inactivate genes in *Xenopus* has been a major drawback in defining endogenous factors necessary (rather than sufficient) for induction. Thus the functions during normal development of *Xwnt-11*, *Vg1*, and *TGFβ-5* RNA localization and of their encoded proteins remain to be determined.

Specification of Cell Fates During Asymmetric Cell Divisions

During *Drosophila* neurogenesis, a stem cell called a neuroblast divides asymmetrically to form a ganglion mother cell (GMC) and another neuroblast. The GMC then divides to form neurons. The Prospero nuclear protein is required for neuronal differentiation (244) and axonal pathfinding (245). The *prospero* mRNA and the Prospero protein are intially apically localized in the neuroblast at interphase but relocalize basally from prophase through telophase, thus segregating into the GMC (127, 246, 247). Basal localization of *prospero* RNA requires Inscuteable and Staufen proteins (127), and Staufen binds directly to the *prospero* 3′-UTR (127). The Miranda protein functions as an adapter that links Prospero protein to the basal cell membrane during the asymmetric

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

neuroblast division (248). Thus RNA and protein localization are used to segregate the Prospero protein into one product of an asymmetric cell division, conferring appropriate neuronal fates upon that cell and its progeny.

Asymmetric segregation of cell fate determinants through mRNA localization has also been described in the budding yeast *S. cerevisiae*. In this case, during cell division the *ASH1* mRNA is localized to the site of the bud and then into the daughter cell that forms there (128, 129). The *ASH1* protein acts as a repressor of the HO endonuclease, which is responsible for mating-type switching (128, 129). Thus localization of the *ASH1* mRNA and its asymmetric segregation into the daughter cell ensures that the daughter cell cannot switch mating type while the mother cell (which does not inherit *ASH1* mRNA) can switch.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

EVOLUTIONARY CONSIDERATIONS

Many features of mRNA localization appear to have been conserved during evolution, suggesting that RNA localization is an ancient mechanism for producing cytoplasmic asymmetry. For example, large stereotypic secondary structures in 3′-UTRs that function in *bicoid* transcript localization are evolutionarily conserved and functionally interchangeable between *Drosophila* species separated by over 60 million years (167). Further, the *bicoid* 3′-UTR, which directs anterior RNA localization in the oocyte, can also direct apical transcript localization in epithelia such as the blastoderm (65). Consistent with this observation, *bicoid* RNA is localized apically in the nurse cells prior to its transport into the oocyte during normal development. Thus, at least within the same species, different polarized cell types appear to share localization signals and factors.

More remarkable is the fact that the mammalian *tau* 3′-UTR, which directs *tau* transcript localization to the axons of neurons, can also direct vegetal transcript localization in *Xenopus* oocytes with a pattern and dynamics indistinguishable from *Vg1* transcripts (158). This result suggests that RNA targeting elements and localization machinery are conserved from *Xenopus* to mammals and from oocytes to neurons. Whether this functional conservation extends to the primary RNA sequence level remains to be seen; however, a small sequence element has been reported to be conserved in the 3′-UTR of *tau*, *Vg1*, and several other localized RNAs in mammals, *Xenopus*, and even *Drosophila* (187).

Recently, RNA localization has been reported in the budding yeast, *S. cerevisiae* (128, 129), and functions during budding to confer asymmetric fates on the mother and daughter cells. mRNA localization (e.g. of *prospero* transcripts) can serve a similar function in higher eukaryotes (127). This suggests that the process of RNA localization dates at least to the invention of single-celled organisms with specialized cytoplasmic domains and/or that undergo asymmetric

cell divisions. The demonstration that the yeast *ASH1* mRNA's 3′-UTR carries information for intracellular targeting implies that the position of *cis*-acting localization elements may be conserved in mRNAs from yeast to mammals. Future studies that focus on the identification and analysis of *trans*-acting factors that target RNAs for localization are likely to uncover additional conserved components of the cytoplasmic RNA localization mechanism.

ACKNOWLEDGMENTS

The following kindly shared unpublished or in press results with us: P Macdonald, R Cohen, P Lasko, W Theurkauf, T Hazelrigg, H Krause, R Long, R Singer, P Takizawa, H Tiedge, L Etkin, and K Mowry. Special thanks to L Etkin, University of Texas, for providing the images of *Xenopus* localized RNAs used in Figure 4. We thank S Lewis, L Etkin, and M Kloc for comments on the manuscript. Our research on RNA localization has been funded over the past decade by research grants to HDL from the National Institutes of Health, the National Science Foundation, and currently the Medical Research Council of Canada.

Visit the *Annual Reviews home page* at http://www.AnnualReviews.org.

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Literature Cited

1. Lipshitz HD, ed. 1995. *Localized RNAs*. Austin, TX: Landes/Springer-Verlag
2. Meller VH, Wu K-H, Roman G, Kuroda MI, Davis RL. 1997. *Cell* 88:445–57
3. Herzing LB, Romer JT, Horn JM, Ashworth A. 1997. *Nature* 386:272–75
4. Penny GD, Kay GF, Sheardown SA, Rastan S, Brockdorff N. 1996. *Nature* 379:131–37
5. Rebagliati MR, Weeks DL, Harvey RP, Melton DA. 1985. *Cell* 42:769–77
6. Frigerio G, Burri M, Bopp D, Baumgartner S, Noll M. 1986. *Cell* 47:735–46
7. Berleth T, Burri M, Thoma G, Bopp D, Richstein S, et al. 1988. *EMBO J.* 7:1749–56
8. Frohnhöfer HG, Nüsslein-Volhard C. 1986. *Nature* 324:120–25
9. King RC. 1970. *Ovarian Development in Drosophila melanogaster*. New York: Academic
10. McKearin D, Ohlstein B. 1995. *Development* 121:2937–47
11. Lin HF, Yue L, Spradling AC. 1994. *Development* 120:947–56
12. Lin HF, Spradling AC. 1995. *Dev. Genet.* 16:6–12
13. de Cuevas M, Lee JK, Spradling AC. 1996. *Development* 122:3959–68
14. Stephenson EC. 1995. See Ref. 1, pp. 63–76
15. Cooley L, Theurkauf WE. 1994. *Science* 266:590–96
16. Ding D, Parkhurst SM, Lipshitz HD. 1993. *Proc. Natl. Acad. Sci. USA* 90:2512–16
17. Yue L, Spradling AC. 1992. *Genes Dev.* 6:2443–54
18. Mahone M, Saffman EE, Lasko PF. 1995. *EMBO J.* 14:2043–55
19. Suter B, Romberg LM, Steward R. 1989. *Genes Dev.* 3:1957–68
20. Mach JM, Lehmann R. 1997. *Genes Dev.* 11:423–35
21. Neuman-Silberberg FS, Schüpbach T. 1993. *Cell* 75:165–74
22. Cheung H-K, Serano TL, Cohen RS. 1992. *Development* 114:653–61
23. Lantz V, Ambrosio L, Schedl P. 1992. *Development* 115:75–88
24. Kim-Ha J, Smith JL, Macdonald PM. 1991. *Cell* 66:23–35
25. Ephrussi A, Dickinson LK, Lehmann R. 1991. *Cell* 66:37–50

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

26. Nakamura A, Amikura R, Mukai M, Kobayashi S, Lasko PF. 1996. *Science* 274:2075–79
27. Golumbeski GS, Bardsley A, Tax F, Boswell RE. 1991. *Genes Dev.* 5:2060–70
28. Wang C, Dickinson LK, Lehmann R. 1994. *Dev. Dyn.* 199:103–15
29. Tirronen M, Lahti V-P, Heino TI, Roos C. 1995. *Mech. Dev.* 52:65–75
30. Hales KH, Meredith JE, Storti RV. 1994. *Dev. Biol.* 165:639–53
31. Theurkauf WE. 1994. *Dev. Biol.* 165: 352–60
32. St. Johnston D, Driever W, Berleth T, Richstein S, Nüsslein-Volhard C. 1989. *Development* 107(Suppl.):13–19
33. Karlin-McGinnes M, Serano TL, Cohen RS. 1996. *Dev. Genet.* 19:238–48
34. Roth S, Neuman-Silberberg FS, Barcelo G, Schüpbach T. 1995. *Cell* 81:967–78
35. González-Reyes A, Elliot H, Johnston DS. 1995. *Nature* 375:654–58
36. Clark IE, Jan LY, Jan YN. 1997. *Development* 124:461–70
37. Pokrywka N-J, Stephenson EC. 1995. *Dev. Biol.* 167:363–70
38. Lane ME, Kalderon D. 1994. *Genes Dev.* 8:2986–95
39. Ruohola H, Bremer KA, Baker D, Swedlow JR, Jan LY, Jan YN. 1991. *Cell* 66:433–49
40. Gillespie DE, Berg CA. 1995. *Genes Dev.* 9:2495–508
41. Clark I, Giniger E, Ruohola-Baker H, Jan LY, Jan YN. 1994. *Curr. Biol.* 4:289–300
42. González-Reyes A, Johnston DS. 1994. *Science* 266:639–41
43. Gavis ER, Curtis D, Lehmann R. 1996. *Dev. Biol.* 176:36–50
44. Zaccai M, Lipshitz HD. 1996. *Dev. Genet.* 19:249–57
45. Zaccai M, Lipshitz HD. 1996. *Zygote* 4: 159–66
46. Deleted in proof
47. Lasko PF. 1994. *Molecular Genetics of* Drosophila *oogenesis*. Austin, TX: Landes
48. Mahajan-Miklos S, Cooley L. 1994. *Cell* 78:291–301
49. Cooley L, Verheyen E, Ayers K. 1992. *Cell* 69:173–84
50. Cant K, Knowles BA, Mooseker MS, Cooley L. 1994. *J. Cell Biol.* 125:369–80
51. Theurkauf WE, Smiley S, Wong ML, Alberts BM. 1992. *Development* 115:923–36
52. Theurkauf WE. 1994. *Science* 265: 2093–96
53. Deleted in proof
54. Karr TL, Alberts BM. 1986. *J. Cell Biol.* 102:1494–509
55. Sullivan W, Fogarty P, Theurkauf W. 1993. *Development* 118:1245–54
56. Wang C, Lehmann R. 1991. *Cell* 66: 637–47
57. Jongens TA, Hay B, Jan LY, Jan YN. 1992. *Cell* 70:569–84
58. Foe VA, Alberts BM. 1983. *J. Cell Sci.* 61:31–70
59. Raff JW, Whitfield WGF, Glover DM. 1990. *Development* 110:1249–61
60. Dalby B, Glover DM. 1992. *Development* 115:989–97
61. Ding D, Lipshitz HD. 1993. *Zygote* 1: 257–71
62. Whitfield WGF, Gonzalez C, Sanchez-Herrero E, Glover DM. 1989. *Nature* 338:337–40
63. Ding D, Parkhurst SM, Halsell SR, Lipshitz HD. 1993. *Mol. Cell. Biol.* 13: 3773–81
64. Edgar BA, O'Dell GM, Schubiger G. 1987. *Genes Dev.* 1:1226–37
65. Davis I, Ish-Horowicz D. 1991. *Cell* 67:927–40
66. Gergen JP, Butler BA. 1988. *Genes Dev.* 2:1179–93
67. Tepass U, Speicher SA, Knust E. 1990. *Cell* 61:787–99
68. Baker NE. 1988. *Dev. Biol.* 125:96–108
69. Macdonald PM, Ingham PW, Struhl G. 1986. *Cell* 47:721–34
70. King ML. 1995. See Ref. 1, pp. 137–48
71. Dumont JN. 1972. *J. Morphol.* 136:153–80
72. Gerhart J, Keller R. 1986. *Annu. Rev. Cell Biol.* 2:201–29
73. Gerhart J, Danilchik M, Doniach T, Roberts S, Rowning B, Stewart R. 1989. *Development* 107(Suppl.):37–51
74. Kloc M, Etkin LD. 1995. See Ref. 1, pp. 149–56
75. Smith JL, Wilson JE, Macdonald PM. 1992. *Cell* 70:849–59
76. Ephrussi A, Lehmann R. 1992. *Nature* 358:387–92
77. Wylie CC, Heasman J, Snape A, O'Driscoll M, Holwill S. 1985. *Dev. Biol.* 112:66–72
78. Mosquera L, Forristall C, Zhou Y, King ML. 1993. *Development* 117:377–86
79. Zhou Y, King M-L. 1996. *Development* 122:2947–53
80. Wylie CC, Brown D, Godsave SF, Quarmby J, Heasman J. 1985. *J. Embryol. Exp. Morphol.* 89(Suppl.):1–15
81. Linnen JM, Bailey CP, Weeks DL. 1993. *Gene* 128:181–88

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

82. Weeks DL, Melton DA. 1987. *Proc. Natl. Acad. Sci. USA* 84:2798–802
83. Hudson JW, Alarcón VB, Elinson RP. 1996. *Dev. Genet.* 19:190–98
84. Otte AP, McGrew LL, Olate J, Nathanson NM, Moon RT. 1992. *Development* 116:141–46
85. Hinkley CS, Martin JF, Leibham D, Perry M. 1992. *Mol. Cell. Biol.* 12:638–49
86. Otte AP, Moon RT. 1992. *Cell* 68:1021–29
87. Kloc M, Reddy BA, Miller M, Eastman E, Etkin L. 1991. *Mol. Reprod. Dev.* 28:341–45
88. Reddy BA, Kloc M, Etkin LD. 1992. *Mech. Dev.* 39:1–8
89. Kloc M, Miller M, Carrasco A, Eastman E, Etkin L. 1989. *Development* 107:899–907
90. Kloc M, Etkin LD. 1995. *Development* 121:287–97
91. Stennard F, Carnac G, Gurdon JB. 1996. *Development* 122:4179–88
92. Zhang J, King M-L. 1996. *Development* 122:4119–29
93. Melton DA. 1987. *Nature* 328:80–82
94. Yisraeli JK, Sokol S, Melton DA. 1990. *Development* 108:289–98
95. Elinson RP, King M-L, Forristall C. 1993. *Dev. Biol.* 160:554–62
96. Pondel MD, King ML. 1988. *Proc. Natl. Acad. Sci. USA* 85:7612–16
97. Kloc M, Spohr G, Etkin LD. 1993. *Science* 262:1712–14
98. Ku M, Melton DA. 1993. *Development* 119:1161–73
99. Zhou Y, King ML. 1997. *Dev. Biol.* 179:173–83
100. Swalla BJ, Jeffery WR. 1996. *Dev. Biol.* 178:23–34
101. Swalla BJ, Jeffery WR. 1995. *Dev. Biol.* 170:353–64
102. Swalla BJ, Jeffrey WR. 1996. *Dev. Genet.* 19:258–67
103. Vlahou A, Gonzalez-Rimbau M, Flytzanis CN. 1996. *Development* 122:521–26
104. Steward O, Kleiman R, Banker G. 1995. See Ref. 1, pp. 235–55
105. Van Minnen J. 1994. *Histochem. J.* 26: 377–91
106. Garner CC, Tucker RP, Matus A. 1988. *Nature* 336:674–76
107. Tiedge H, Fremeau RT, Weinstock PH, Arancio O, Brosius J. 1991. *Proc. Natl. Acad. Sci. USA* 88:2093–97
108. Tiedge H, Chen W, Brosius J. 1993. *J. Neurosci.* 13:2382–90
109. Mayford M, Baranes D, Podsypanina K, Kandel ER. 1996. *Proc. Natl. Acad. Sci. USA* 93:13250–55
110. Furuichi T, Simon-Chazottes D, Fujino I, Yamada N, et al. 1993. *Recept. Channels* 1:11–24
111. Tiedge H, Zhou A, Thorn NA, Brosius J. 1993. *J. Neurosci.* 13:4214–19
112. Litman P, Barg J, Rindzoonski L, Ginzburg I. 1993. *Neuron* 10:627–38
113. Hannan AJ, Schevzov G, Gunning P, Jeffrey PL, Weinberger RP. 1995. *Mol. Cell. Neurosci.* 6:397–411
114. Murphy D, Levy A, Lightman S, Carter D. 1989. *Proc. Natl. Acad. Sci. USA* 86: 9002–5
115. Jirikowski GF, Sanna PP, Bloom FE. 1990. *Proc. Natl. Acad. Sci. USA* 87: 7400–4
116. Mohr E, Fehr S, Richter D. 1991. *EMBO J.* 10:2419–24
117. Mohr E, Richter D. 1992. *Eur. J. Neurosci.* 9:870–76
118. Ressler KJ, Sullivan SL, Buck LB. 1994. *Cell* 79:1245–55
119. Vassar R, Chao SK, Sitcheran R, Nuñez JM, Vosshall LB, Axel R. 1994. *Cell* 79:981–91
120. Trapp BD, Moench T, Pully M, Barbosa E, Tennekoon G, Griffin J. 1987. *Proc. Natl. Acad. Sci. USA* 84:7773–77
121. Colman DR, Kriebich G, Frey AB, Sabatini DD. 1982. *J. Cell Biol.* 95:598–608
122. Laitala-Leinonen T, Howell ML, Dean GE, Väänänen HK. 1996. *Mol. Biol. Cell* 7:129–42
123. Sundell CL, Singer RH. 1991. *Science* 253:1275–77
124. Lawrence JB, Singer RH. 1986. *Cell* 45:407–15
125. Cheng H, Bjerknes M. 1989. *J. Mol. Biol.* 210:541–49
126. Banerjee U, Renfranz PJ, Pollock JA, Benzer S. 1987. *Cell* 49:281–91
127. Li P, Yang XH, Wasser M, Cai Y, Chia W. 1997. *Cell* 90:437–47
128. Long RM, Singer RH, Meng XH, Gonzalez I, Nasmyth K, Jansen R-P. 1997. *Science* 277:383–87
129. Takizawa P, Sil A, Swedlow JR, Herskowitz I, Vale RD. 1997. *Nature* 389: 90–93
130. Daneholt B. 1997. *Cell* 88:585–88
131. Francis-Lang H, Davis I, Ish-Horowicz D. 1996. *EMBO J.* 15:640–49
132. Kobayashi S, Okada M. 1990. *Nucleic Acids Res.* 18:4592
133. Kobayashi S, Okada M. 1989. *Development* 107:733–42
134. Kobayashi S, Amikura R, Okada M. 1993. *Science* 260:1521–24
135. Kobayashi S, Amikura R, Okada M. 1994. *Int. J. Dev. Biol.* 38:193–99

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

136. Kobayashi S, Amikura R, Nakamura A, Saito H, Okada M. 1995. *Dev. Biol.* 169: 384–86
137. Ding D, Whittaker KL, Lipshitz HD. 1994. *Dev. Biol.* 163:503–15
138. Gottlieb E. 1990. *Curr. Opin. Cell Biol.* 2:1080–86
139. Gavis ER, Lehmann R. 1994. *Nature* 369:315–18
140. Dahanukar A, Wharton RP. 1996. *Genes Dev.* 10:2610–20
141. Smibert CA, Wilson JE, Kerr K, Macdonald PM. 1996. *Genes Dev.* 10:2600–9
142. Halsell SR, Lipshitz HD. 1995. See Ref. 1, pp. 9–39
143. Deleted in proof
144. Cooperstock RL, Lipshitz HD. 1998. *Semin. Cell Dev. Biol.* 8(6):541–49
145. Li MG, McGrail M, Serr M, Hays TS. 1994. *J. Cell Sci.* 126:1475–94
146. McGrail M, Hays TS. 1997. *Development* 124:2409–19
147. Pokrywka N-J, Stephenson EC. 1994. *Dev. Biol.* 166:210–19
148. Suter B, Steward R. 1991. *Cell* 67:917–26
149. Lantz V, Chang JS, Horabin JI, Bopp D, Schedl P. 1994. *Genes Dev.* 8:598–613
150. Pokrywka NJ, Stephenson EC. 1991. *Development* 113:55–66
151. Macdonald PM, Kerr K. 1997. *RNA* 3: 1413–1420
152. Erdélyi M, Michon A-M, Guichet A, Glozter JB, Ephrussi A. 1995. *Nature* 377:524–27
153. Tetzlaff MT, Jäckle H, Pankratz MJ. 1996. *EMBO J.* 15:1247–54
154. Deshler JO, Highett MI, Schnapp BJ. 1997. *Science* 276:1128–31
155. Knowles RB, Sabry JH, Martone ME, Deerinck TJ, Ellisman MH, et al. 1996. *J. Neurosci.* 16:7812–20
156. Ainger K, Avossa D, Morgan F, Hill SJ, Barry C, et al. 1993. *J. Cell Biol.* 123:431–41
157. Behar L, Marx R, Sadot E, Barg J, Ginzburg I. 1995. *Int. J. Dev. Neurosci.* 13:113–27
158. Litman P, Behar L, Elisha Z, Yisraeli JK, Ginzburg I. 1996. *Dev. Biol.* 176:80–94
159. Glotzer JB, Saffrich R, Glotzer M, Ephrussi A. 1997. *Curr. Biol.* 7:326–37
160. Swan A, Suter B. 1996. *Development* 122:3577–86
161. Kloc M, Etkin LD. 1994. *Science* 265: 1101–3
162. Wang SX, Hazelrigg T. 1994. *Nature* 369:400–3
163. Ferrandon D, Elphick L, Nüsslein-Volhard C, St. Johnston D. 1994. *Cell* 79:1221–32
164. Ferrandon D, Koch I, Westhof E, Nüsslein-Volhard C. 1997. *EMBO J.* 16: 1751–58
165. Walter P, Johnson AE. 1994. *Annu. Rev. Cell Biol.* 10:87–119
166. Macdonald PM, Struhl G. 1988. *Nature* 336:595–98
167. Macdonald PM. 1990. *Development* 110:161–72
168. Kim-Ha J, Webster PJ, Smith JL, Macdonald PM. 1993. *Development* 119: 169–78
169. Lantz V, Schedl P. 1994. *Mol. Cell Biol.* 14:2235–42
170. Mowry KL, Melton DA. 1992. *Science* 255:991–94
171. Kislauskis EH, Zhu X-C, Singer RH. 1994. *J. Cell Biol.* 127:441–51
172. Jackson RJ. 1993. *Cell* 74:9–14
173. Muslimov IA, Santi E, Homel P, Perini S, Higgins D, Tiedge H. 1997. *J. Neurosci.* 17:4722–33
174. Whittaker KL, Lipshitz HD. 1995. See Ref. 1, pp. 41–61
175. Kislauskis EH, Li ZF, Singer RH, Taneja KL. 1993. *J. Cell Biol.* 123:165–72
176. Macdonald PM, Kerr K, Smith JL, Leask A. 1993. *Development* 118:1233–43
177. Macdonald PM, Leask A, Kerr K. 1995. *Proc. Natl. Acad. Sci. USA* 92:10787–91
178. St. Johnston D, Brown NH, Gall JG, Jantsch M. 1992. *Proc. Natl. Acad. Sci. USA* 89:10979–83
179. St. Johnston D, Beuchle D, Nüsslein-Volhard C. 1991. *Cell* 66:51–63
180. Ding D, Lipshitz HD. 1993. *BioEssays* 10:651–58
181. Serano T, Cohen R. 1995. *Development* 121:3809–18
182. Dalby B, Glover DM. 1993. *EMBO J.* 12:1219–27
183. Gautreau D, Cote CA, Mowry KL. 1997. *Development* 124:5013–20
184. Kim-Ha J, Kerr K, Macdonald PM. 1995. *Cell* 81:403–12
185. Gavis ER, Lunsford L, Bergsten SE, Lehmann R. 1996. *Development* 122: 2791–800
186. Webster PJ, Liang L, Berg CA, Lasko P, Macdonald PM. 1997. *Genes Dev.* 11:2510–21
187. Yisraeli JK, Oberman F, Pressman Schwartz S, Havin L, Elisha Z. 1995. See Ref. 1, pp. 157–71
188. Schüpbach T, Wieschaus E. 1986. *Roux's Arch. Dev. Biol.* 195:302–17
189. Frohnhöfer HG, Nüsslein-Volhard C. 1987. *Genes Dev.* 1:880–90

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

190. Rongo C, Gavis E, Lehmann R. 1995. *Development* 121:2737–46
191. Bandziulis R, Swanson MS, Dreyfuss G. 1989. *Genes Dev.* 3:431–37
192. Schüpbach T, Wieschaus E. 1991. *Genetics* 129:1119–36
193. Castrillon DH, Gönczy P, Alexander S, Rawson R, Eberhart CG, et al. 1993. *Genetics* 135:489–505
194. Lasko PF, Ashburner M. 1988. *Nature* 335:611–16
195. Hay B, Jan LY, Jan YN. 1988. *Cell* 55: 577–87
196. Liang L, Diehl-Jones W, Lasko P. 1994. *Development* 120:1201–11
197. Schwartz SP, Aisenthal L, Elisha Z, Oberman F, Yisraeli JK. 1992. *Proc. Natl. Acad. Sci. USA* 89:11895–99
198. Perry-O'Keefe H, Kintner C, Yisraeli J, Melton DA. 1990. In *In situ Hybridization and the Study of Development and Differentiation*, ed. N Harris, D Wilkenson, pp. 115–30. Cambridge: Cambridge Univ. Press
199. Elisha Z, Havin L, Ringel I, Yisraeli JK. 1995. *EMBO J.* 14:5109–14
200. Mowry KL. 1996. *Proc. Natl. Acad. Sci. USA* 93:14608–13
201. Marcey D, Watkins WS, Hazelrigg T. 1991. *EMBO J.* 10:4259–66
202. Macdonald PM, Luk SKS, Kilpatrick M. 1991. *Genes Dev.* 5:2455–66
203. Chao Y-C, Donahue KM, Pokrywka NJ, Stephenson EC. 1991. *Dev. Genet.* 12: 333–41
204. Hegdé J, Stephenson EC. 1993. *Development* 119:457–70
205. Webster P, Suen J, Macdonald P. 1994. *Development* 120:2027–37
206. Lasko PF, Ashburner M. 1990. *Genes Dev.* 4:905–21
207. Hay B, Ackerman L, Barbel S, Jan LY, Jan YN. 1988. *Development* 103:625–40
208. Hay B, Jan LY, Jan YN. 1990. *Development* 109:425–33
209. Yoon C, Kawakami K, Hopkins N. 1997. *Development* 124:3157–66
210. Christerson LB, McKearin DM. 1994. *Genes Dev.* 8:614–28
211. Kelley RL. 1993. *Genes Dev.* 7:948–60
212. Serano TL, Karlin-McGinnes M, Cohen RS. 1995. *Mech. Dev.* 51:183–92
213. Prost E, Derychere F, Roos C, Haenlin M, Pantesco V, Mohier E. 1988. *Genes Dev.* 2:891–900
214. Wilson JE, Connell JE, Schlenker JD, Macdonald PM. 1996. *Dev. Genet.* 19: 199–209
215. Rittenhouse KR, Berg CA. 1995. *Development* 121:3032–33
216. Wilson JE, Connell JE, Macdonald PM. 1996. *Development* 122:1631–39
217. St. Johnston D. 1995. *Cell* 81:161–70
218. Neuman-Silberberg FS, Schupbach T. 1994. *Development* 120:2457–63
219. Driever W, Nüsslein-Volhard C. 1988. *Cell* 54:83–93
220. Driever W, Nüsslein-Volhard C. 1988. *Cell* 54:95–104
221. Struhl G, Struhl K, Macdonald PM. 1989. *Cell* 57:1259–73
222. Driever W, Thoma G, Nüsslein-Volhard C. 1989. *Nature* 340:363–67
223. Driever W, Nüsslein-Volhard C. 1989. *Nature* 337:138–43
224. Driever W, Siegel V, Nüsslein-Volhard C. 1990. *Development* 109:811–20
225. Wharton RP, Struhl G. 1991. *Cell* 67: 955–67
226. Rivera-Pomar R, Niessing D, Schmidt-Ott U, Gehring WJ, Jäckle H. 1996. *Nature* 379:746–49
227. Dubnau J, Struhl G. 1996. *Nature* 379: 694–99
228. Mlodzik M, Fjose A, Gehring WJ. 1985. *EMBO J.* 4:2961–69
229. Macdonald PM, Struhl G. 1986. *Nature* 324:537–45
230. Lehmann R, Nüsslein-Volhard C. 1991. *Developmen* 112:679–93
231. Gavis ER, Lehmann R. 1992. *Cell* 71: 301–13
232. Hülskamp M, Schröder C, Pfeifle C, Jäckle H, Tautz D. 1989. *Nature* 338: 629–32
233. Irish V, Lehmann R, Akam M. 1989. *Nature* 338:646–48
234. Lehmann R, Nüsslein-Volhard C. 1987. *Dev. Biol.* 119:402–17
235. Tautz D, Lehmann R, Schnürch H, Schuh R, Seifert E, et al. 1987. *Nature* 327:383–89
236. Stanojevic D, Hoey T, Levine M. 1989. *Nature* 341:331–35
237. Lehmann R, Nüsslein-Volhard C. 1987. *Nature* 329:167–70
238. Lehmann R, Nüsslein-Volhard C. 1986. *Cell* 47:141–52
239. Jongens TA, Ackerman LD, Swedlow JR, Jan LY, Jan YN. 1994. *Genes Dev.* 8:2123–36
240. Kobayashi S, Yamada M, Asaoka M, Kitamura T. 1996. *Nature* 380:708–11
241. Thomsen GH, Melton DA. 1993. *Cell* 74:433–41
242. Dale L, Matthews G, Colman A. 1993. *EMBO J.* 12:4471–80
243. Kondaiah P, Sands M, Smith JM, Fields A, Roberts AB, et al. 1990. *J. Biol. Chem.* 265:1089–93

244. Doe CQ, Chu-LaGraff Q, Wright DM, Scott MP. 1991. *Cell* 65:451–64
245. Vaessin H, Grell E, Wolff E, Bier E, Jan LY, Jan YN. 1991. *Cell* 67:941–53
246. Hirata J, Nakagoshi H, Nabeshima Y-I, Matsuzuka F. 1995. *Nature* 377:627–30
247. Knoblich JA, Jan LY, Jan YN. 1995. *Nature* 377:624–27
248. Shen C-P, Jan LY, Jan YN. 1997. *Cell* 90:449–58
249. Jeffery WR, Tomlinson CR, Brodeur RD. 1983. *Dev. Biol.* 99:408–20
250. Macdonald PM. 1992. *Development* 114:221–32
251. Aït-Ahmed O, Thomas-Cavallin M, Rosset R. 1987. *Dev. Biol.* 122:153–62
252. Pomeroy ME, Lawrence JB, Singer RH, Billings-Gagliardi S. 1991. *Dev. Biol.* 143:58–67
253. Trembleau A, Calas A, Fèvre-Montange M. 1990. *Mol. Brain Res.* 8:37–45
254. Perry BA. 1988. *Cell Differ. Dev.* 25:99–108

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

Annual Review of Biochemistry
Volume 67, 1998

CONTENTS

An Accidental Biochemist, *Edwin G. Krebs*	0
HIV-1: Fifteen Proteins and an RNA, *Alan D. Frankel and John A. T. Young*	1
Sphingolipid Functions in *Saccharomyces Cerevisiae* : Comparison to Mammals, *Robert C. Dickson*	27
Transporters of Nucleotide Sugars, ATP, and Nucleotide Sulfate in the Endoplasmic Reticulum and Golgi Apparatus, *Carlos B. Hirschberg, Phillips W. Robbins, and Claudia Abeijon*	49
Ribonucleotide Reductases, *A. Jordan and P. Reichard*	71
Modified Oligonucleotides: Synthesis and Strategy for Users, *Sandeep Verma and Fritz Eckstein*	99
The Molecular Control of Circadian Behavioral Rhythms and Their Entrainment in *Drosophila, Michael W. Young*	135
Ribonuclease P: Unity and Diversity in a tRNA Processing Ribozyme, *Daniel N. Frank and Norman R. Pace*	153
Base Flipping, *Richard J. Roberts and Xiaodong Cheng*	181
The Caveolae Membrane System, *Richard G. W. Anderson*	199
How Cells Respond to Interferons, *George R. Stark, Ian M. Kerr, Bryan R. G. Williams, Robert H. Silverman, and Robert D. Schreiber*	227
Nucleocytoplasmic Transport: The Soluble Phase, *Iain W. Mattaj and Ludwig Englmeier*	265
Role of Small G Proteins in Yeast Cell Polarization and Wall Biosynthesis, *Enrico Cabib, Jana Drgonová, and Tomás Drgon*	307
RNA Localization in Development, *Arash Bashirullah, Ramona L. Cooperstock, and Howard D. Lipshitz*	335
Biochemistry and Genetics of von Willebrand Factor, *J. Evan Sadler*	395
The Ubiquitin System, *Avram Hershko and Aaron Ciechanover*	425
Phosphoinositide Kinases, *David A. Fruman, Rachel E. Meyers, and Lewis C. Cantley*	481
The Green Fluorescent Protein, *Roger Y. Tsien*	509
Alteration of Nucleosome Structure as a Mechanism of Transcriptional Regulation, *J. L. Workman, and R. E. Kingston*	545
Structure and Function in GroEL-Mediated Protein Folding, *Paul B. Sigler, Zhaohui Xu, Hays S. Rye, Steven G. Burston, Wayne A. Fenton, and Arthur L. Horwich*	581
Matrix Proteoglycans: From Molecular Design to Cellular Function, *Renato V. Iozzo*	609
G Protein Coupled Receptor Kinases, *Julie A. Pitcher, Neil J. Freedman, and Robert J. Lefkowitz*	653
Enzymatic Transition States and Transition State Analog Design, *Vern L. Schramm*	693

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.

The DNA Replication Fork in Eukaryotic Cells, *Shou Waga and Bruce Stillman* 721

TGF-beta Signal Transduction, *J. Massagué* 753

Pathologic Conformations of Prion Proteins, *Fred E. Cohen and Stanley B. Prusiner* 793

The AMP-Activated/SNF1 Protein Kinase Subfamily: Metabolic Sensors of the Eukaryotic Cell?, *D. Grahame Hardie, David Carling, and Marian Carlson* 821

Annu. Rev. Biochem. 1998.67:335-394. Downloaded from arjournals.annualreviews.org
by CALIFORNIA INSTITUTE OF TECHNOLOGY on 09/08/05. For personal use only.