The EMBO Journal Vol. 20 No. 15 pp. 3984–3992, 2001

Roles for 147 embryonic lethal genes on *C.elegans* chromosome I identified by RNA interference and video microscopy

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Early embryonic development involves complex events such as the regulation of cell division and the establishment of embryonic polarity. To identify genes involved in these events, we collected four-dimensional time-lapse video recordings of the first three cell divisions and analysed terminal phenotypes after RNA interference of 147 embryonic lethal genes previously identified in a systematic screen of *Caenorhabditis elegans* chromosome I. Over half gave defects in early processes such as meiosis, the assembly or position of the first mitotic spindle, cytokinesis, and proper nuclear positioning. For some phenotypic classes, the majority of genes are involved in a shared biochemical process. In addition, we identified loss-of-function phenotypes for genes of unknown function, but for which homologues exist in other organisms, shedding light on the function of these uncharacterized genes. When applied to the whole genome, this approach should identify the vast majority of genes required for early cell processes, paving the way for a greatly improved understanding of these processes and their regulation at the molecular level.

Keywords: *Caenorhabditis elegans*/embryogenesis/RNAi/video recordings

Introduction

In *Caenorhabditis elegans*, early embryonic development follows a stereotypical series of events that is essentially invariant in wild-type animals (reviewed in Kemphues and Strome, 1997; illustrated in Figure 1A–I). These include the completion of meiosis following fertilization, pronuclear migration, spindle assembly, asymmetric positioning of the spindle in the zygote, cytokinesis, and blastomere-specific cell cycle timing and establishment of correct spindle orientation in the subsequent cell divisions. Although some of the genes required for these early events are known, the majority of those involved have yet to be discovered. These early events can be observed using time-lapse video microscopy, and the reproducibility of wild-type development allows the identification of relatively subtle defects in mutants. These features make *C.elegans* an excellent system for studying basic processes such as cell division and the establishment of cell polarity.

The *C.elegans* genomic sequence is essentially complete, with 19 099 genes predicted (The *C. elegans* Sequencing Consortium, 1998). This information, coupled with the technique of RNA interference (RNAi), make it possible to ask which genes have a role in early embryonic development. RNAi causes the mRNA of the gene of interest to be specifically degraded following introduction of the corresponding double-stranded RNA into the animal, making it an ideal method for investigating gene function (Fire *et al*., 1998; Montgomery *et al*., 1998). We previously made a bacterial library for RNAi of ~90% of *C.elegans* chromosome I genes and identified those required for embryonic development (Fraser *et al*., 2000). Here we present a phenotypic analysis of 147 of these genes. These results provide a starting point for future studies of processes such as meiosis, mitosis, cytokinesis and cell polarity.

Results

Identifying chromosome I genes required for early development

To identify genes involved in early embryonic processes, we analysed 147 genes that we previously showed to have a strong embryonic lethal phenotype using RNAi (Fraser *et al*., 2000). After RNAi, we video recorded embryonic development up to the eight-cell stage at least twice for each gene, and analysed these recordings for any deviation from wild-type development. After video recording, embryos were left to develop until the terminal stage, and the resulting phenotype was analysed visually for completion of embryonic cell divisions and for the extent of differentiation. Complete results are given in Table I, and video recordings are available at http://www.wormbase.org

We identified a wide range of defects, and assigned these to 17 distinct phenotypic classes (Figure 1; Table I). Over 50% of the genes analysed gave an RNAi defect detectable in the first three cell cycles, including defects in meiosis, the assembly or positioning of the mitotic spindle, cytokinesis, and the position or morphology of the nuclei (Figures 1 and 2).

For some phenotypic classes, most of the genes identified are involved in a shared biochemical process. For example, 8 of 10 genes having a one-cell arrest RNAi phenotype encode components of either the proteasome or the anaphase promoting complex (APC) (Figure 1O; Table I). This cell cycle arrest is consistent with their known function in the regulation of cell cycle proteins; previous study of one component of the APC complex, EMB-30, yielded a similar phenotype (Zachariae and Nasmyth, 1999; Furuta *et al*., 2000). A second striking example is the Pronuclear Envelope phenotypic class, in which the pronuclear and subsequent nuclear envelopes

have an indistinct appearance (Figure 1Q). The majority of genes in this class encode components of the nuclear envelope or are involved in nuclear transport (e.g. a nuclear lamin and karyopherin; Table I), suggesting that this phenotype is due to an improperly formed nuclear envelope. A third example is the Nuclear Morphology class. Many genes in this class encode proteins involved in DNA synthesis (e.g. DNA primase; Table I) or proteins involved in chromatin regulation or condensation (e.g. histone H2A; Table I). Thus, improper packaging or synthesis of DNA results in abnormal nuclear morphology. Applying this information to previously identified mutants with the above phenotypes (e.g. Gönczy *et al*., 1999) should facilitate their cloning by a candidate gene approach.

We identified many other genes with early RNAi phenotypes that provide novel functional information about previously studied genes or pathways. For example, we found that RNAi of the *C.elegans* homologue of glycogen synthase kinase 3 (*gsk-3*), a component of the Wnt pathway, produces embryos with a spindle orientation defect in the P2 cell (Figure 1R). Previous studies of *gsk-3* did not identify this phenotype, but work on *gsk-3* and other components of the Wnt pathway has revealed a requirement for the orientation of a different cell division (Schlesinger *et al*., 1999). It may be that the Wnt pathway has a more general role in orienting mitotic spindles than previously thought, or it might be important for the fate of the P2 cell. One gene we identified in the Cytokinesis class is similar to the yeast gene *SCD6*, a suppressor of clathrin deficiency. The yeast function is consistent with a role in the regulation of vesicle trafficking, and work in *C.elegans* has suggested that vesicle fusion is necessary for completion of the cytokinetic furrow (Nelson *et al*., 1996; Jantsch-Plunger and Glotzer, 1999); the RNAi phenotype of the *C.elegans SCD6* homologue supports this view. In the Spindle Position class, we identified a homologue of prefoldin 6 (human KE2/yeast GIM1/YEK2). Previous work in yeast suggested that this protein might be involved in promoting the formation of functional α- and γ-tubulin (Geissler *et al*., 1998).

We also identified a number of genes for which no function had previously been described (Table I). In many cases, homologues exist in other animals and our data provide an entry point for studying these novel conserved genes. Furthermore, some phenotypic classes predict the biochemical process involved (e.g. Nuclear Morphology and packaging or synthesis of DNA); thus, a possible biochemical function can be suggested for unknown proteins in these classes.

Terminal arrest phenotypes

Genes that did not give an RNAi phenotype detectable in the first three divisions were placed into one of three phenotypic classes based on their terminal phenotype (Table I; Figure 3). Genes in the Late Embryonic Phenotype (LEP) class had a consistent, reproducible terminal RNAi phenotype, those in the Variable class had a range of different arrest phenotypes, and those in the Slow Development class developed much slower than wild-type embryos, but otherwise appeared normal. Genes in this latter class are involved in a range of basal cell processes, but half of the genes in the Variable class encode components of the translation machinery, including five ribosomal proteins and a tRNA synthetase (Table I). After RNAi of these genes, general production of both maternal and zygotic proteins is likely to be reduced, and this may result in embryos with a variable amount of a range of different proteins, resulting in a variable arrest. In addition, RNAi of genes involved in translation occasionally resulted in multiple female pronuclei or multiple nuclei during cleavages, suggesting a possible defect in meiosis and/or mitosis (Table I, Notes column).

The LEP class is large and diverse, comprising genes that are involved in a range of different processes. The LEP class can be subdivided according to terminal phenotype, which in some cases can provide a possible indication of the function of a gene. For example, previous work has shown that mutants that fail to elongate past the 2-fold stage have defects either in the epidermis or in the underlying muscles (Williams and Waterston, 1994; Wissmann *et al*., 1997; Costa *et al*., 1998). In the 1- to 2-fold arrest class, we identified two genes involved in epidermal morphogenesis and for which mutants were previously shown to have this phenotype: *hmp-2*, which encodes a β-catenin, and *let-502*, related to Rho-binding kinases (Williams and Waterston, 1994; Wissmann *et al*., 1997; Costa *et al*., 1998). Based on the terminal arrest phenotype, some of the other genes in this class might be involved in muscle or epidermal structure or function (e.g. F53B8.1; Figure 3F). Other terminal arrest phenotypes (e.g. Enclosed) are less informative and comprise genes that clearly have a wide range of different functions.

Comparison of phenotype to predicted biochemical function

Further information on the type of gene involved in different cell processes can be gained from examination of the predicted cellular roles of the genes within each phenotypic class [the biochemical functional classes are defined in Fraser *et al*. (2000); Figure 2]. Of the genes having an early RNAi phenotype, the largest biochemical functional class contains proteins involved in cell biological and cell architecture processes—this includes components of the cytoskeleton and genes involved in vesicle fusion (Figure 2). In contrast, the largest class of genes with a LEP is RNA synthesis or processing (Figure 2A).

In wild-type embryos, tissue differentiation normally occurs when cell divisions are essentially complete (~500 cells); we therefore subdivided the LEP class into those where embryos arrest prior to the 500-cell stage with no differentiation (LEP <500 cells) and those with >500 cells and obvious tissue differentiation (LEP >500 cells). The LEP <500 cell genes account for the majority of those involved in basal transcription or mRNA splicing (Figure 2). Normal early development of these is consistent with previous work showing that inhibitors of transcription allow embryonic development up to the beginning of gastrulation (Edgar *et al*., 1994; Powell-Coffman *et al*., 1996). Among the LEP >500 cell genes, the largest functional class contains proteins involved in specific transcription, such as transcription factors. This class includes previously studied genes such as *pop-1*, a

Table I. Phenotypic classification of 147 *C.elegans* chromosome I *emb* genes

GenePair	2 genes same gene	>80%	Terminal Phenotype	Functional Class	Locus	Description	Ooc	Sperm	GL intr	CE	DM	SC	HS	Notes
1-Cell Arrest														
B0511.9			<200 cells	Unknown										escapers have spindle orientation defects
C36B1.4			1-12 cell arrest	Prot Syn/Deg		proteasome subunit				CE	DM	SC	HS	
F25H2.9			1-12 cell arrest	Prot Syn/Deg		proteasome subunit				CE	DM	SC	HS	first spindle is made
F39H11.5			1-12 cell arrest	Prot Syn/Deg		proteasome subunit					DM	SC	HS	
F56H1.4			1-12 cell arrest	Prot Syn/Deg		proteasome subunit			x	CE	DM	SC	HS	
K05C4.1			1-12 cell arrest	Prot Syn/Deg		proteasome subunit				ce	dm	SC	HS	
R12E2.3			1-12 cell arrest	Prot Syn/Deg		proteasome subunit					dm	sc	hs	
T20F5.2			1-12 cell arrest	Prot Syn/Deg		proteasome subunit					DM	SC		
Y110A7A.d			1-12 cell arrest	Cell Cyc/Chrm		cdc27 homologue					dm	sc	hs	
Y47G6A_247.g			1-12 cell arrest	-		2 genes: ATPase like yeast AFG3 / unknown								
Meiosis														
F57B10.12			Differentiated, not enclosed	Cell Cyc/Chrm	*mei-2*	human p60 katanin homologue				CE				multiple female pronuclei
T01G9.5			Differentiated, not enclosed	Cell Cyc/Chrm	*mei-1*	AAA ATPase	x			ce	dm	sc	hs	multiple female pronuclei
C41G7.2			Enclosed	Cell Cyc/Chrm	*klp-16*	claret subfamily kinesin			x	CE	DM	sc	HS	multiple or no female pronuclei
M01E11.6			Enclosed	Cell Cyc/Chrm	*klp-15*	claret subfamily kinesin				CE	DM	sc	hs	multiple female pronuclei
C45G3.1			1-2fold	Cell Bio/Arch		actinin-type actin-binding domain			x		dm			multiple female pronuclei
Pronuclear Envelope														
F26B1.3			<200 cells	Cell Bio/Arch		karyopherin; nuclear import			x	CE	DM	SC	HS	P1 division late; furrow regression
F53F10.5			<200 cells	Cell Bio/Arch		nucleoporin homology								P1 division late
F56A3.3			<200 cells	Unknown		coiled coils								P1 division late
R05D11.3			<200 cells	Cell Bio/Arch		nuclear transport factor 2					DM	SC	HS	P1 division late
T19B4.2			<200 cells	Cell Bio/Arch		possible nuclear pore protein								P1 division late
Y48G1A_54.b			<200 cells	Cell Bio/Arch		karyopherin-beta orthologue					DM	SC	hs	P1 division late
Y48G1A_54.c			<200 cells	Cell Bio/Arch		karyopherin-beta orthologue					DM	SC	hs	P1 division late
DY3.2			Differentiated, not enclosed	Cell Bio/Arch		nuclear type B lamin								
Pronuclear Migration														
T06G6.9			<200 cells	Prot Syn/Deg		prefoldin subunit 3					DM	SC		partial migration
T21E12.4			<200 cells	Cell Bio/Arch	*dhc-1*	dynein heavy chain				ce	DM	SC	hs	no migration
Spindle Assembly														
F32H2.3			1-12 cell arrest	Unknown					x					small abnormal spindle
F26E4.8			1-12 cell arrest	Cell Bio/Arch	*tba-1*	tubulin alpha				CE	DM	SC	HS	no spindle
C47B2.3			<200 cells	Cell Bio/Arch	*tba-2*	tubulin alpha				CE	DM	SC	HS	small abnormal spindle
F56A3.4			<200 cells	Unknown		coiled coils			x	CE	DM	SC	HS	small abnormal spindle
Y39G10A_246.j			200-500 cells	Unknown		coiled coils								short spindle
Y39G10A_246.k			200-500 cells	Unknown										short spindle
F39B2.10			Enclosed	Prot Syn/Deg		DnaJ domain				CE	DM	SC	HS	weak spindle
Spindle Orientation														
T08G11.4			200-500 cells	Unknown		SAM domain						sc	hs	abn cytopl; lg nuclei; ABn orient. abn; slow
F36H2.1			Differentiated, not enclosed	Cell Bio/Arch		P-type ATPase				CE	DM	SC	hs	ABa d/v; P1 sl. l/r
F46A9.4			Differentiated, not enclosed	Prot Syn/Deg		SKP1 family member			x	CE	DM	sc	hs	AB skewed; P1 sometimes fails to rotate
C26C6.2			Enclosed	Signalling	*goa-1*	G-alpha subunit				CE	DM	SC	HS	partial or no rotation in P1; ABa d/v
Y18D10A.5			Enclosed	Signalling	*gsk-3*	GSK-3beta homologue				CE	DM	SC	HS	EMS and P2 orientation incorrect
Y106G6E.6			200-500 cells	Signalling		Casein Kinase 1				CE	DM	sc	HS	div nearly synch; no P1 rot; spindles rock
Spindle Position														
Y110A7A.f			<200 cells	-		2 genes: proteasome subunit / unknown								too posterior; some emb 1 cell arrest
F21C3.5			Differentiated, not enclosed	Cell Bio/Arch		prefoldin subunit 6; Yke2p-homologue					DM	sc		unstable
F22D6.6			Differentiated, not enclosed	Unknown										too posterior
ZK858.4			Differentiated, not enclosed	Unknown	*mel-26*	BTB domain				CE	DM		HS	unstable and posterior
Spindle Position/Orientation														
F20G4.3			Differentiated, not enclosed	Cell Bio/Arch	*nmy-2*	non-muscle myosin-2			x					symmetric
T26E3.3			Differentiated, not enclosed	Cell Bio/Arch	*par-6*	PDZ domain					dm		hs	symmetric
Y110A7A.g			Differentiated, not enclosed	Unknown	*chp-1*	CHORD-domain containing protein					DM		hs	symmetric
Cytokinesis														
T23G11.2			1-12 cell arrest	Energ/Metab		phosphoglucosamine acetyltransferase				ce	dm	sc	hs	no furrow; extra fem. pronuclei; swollen
Y18D10A.20			1-12 cell arrest	Cell Bio/Arch	*pfn-1*	profilin								no furrow
Y39G10A_246.i			1-12 cell arrest	Cell Cyc/Chrm	*icp-1*	INCENP-like; centromeric protein								furrow regr; abnorm fem. pronuc.
F26A3.3			<200 cells	RNA Syn/Proc	*ego-1*	RNA-directed RNA polymerase								extra furrows
Y18D10A.17			<200 cells	Cell Bio/Arch		sim to yeast SCD6, supp. of clathrin deficiency					dm	sc	hs	furrow regresses
Multiple Nuclei														
F56C11.5			<200 cells	Unknown										
T03F1.9			<200 cells	Cell Bio/Arch		human CENP-E like								
F26E4.1			200-500 cells	Signalling	*sur-6*	PP2A regulatory subunit					DM	SC	hs	asters are huge
R06C7.8			Differentiated, not enclosed	Cell Cyc/Chrm		Bub1-like kinase			x	ce	dm	sc		
Nuclear Position														
C53H9.2			Differentiated, not enclosed	Cell Cyc/Chrm		GTPase of unknown function				ce	DM	SC	hs	unstable
Y65B4B_10.b			1-2fold	Prot Syn/Deg		nascent polypeptide associated a-chain					DM	sc	HS	unstable
Y65B4B_10.d			1-2fold	Prot Syn/Deg		nascent polypeptide associated a-chain					DM	sc	HS	unstable
Nuclear Morphology														
F33H2.5			<200 cells	DNA Syn		DNA pol epsilon subunit						SC		
F55A3.7 (a)			<200 cells	RNA Syn/Proc		2 genes: both gen. Chrom. fact. SPT16/CDC68			x					multiple nuclei
K06A5.4			<200 cells	Unknown		coiled coils			x					multiple nuclei
T23D8.6			<200 cells	RNA Syn/Proc		histone H2A								
W02D9.1			<200 cells	DNA Syn		DNA primase (DNA pol alpha subunit)			x	CE		SC	hs	P1 divides too late
Y39G10A_246.e			<200 cells	DNA Syn		CDC21/replication licensing factor MCM4								
R06C7.5			Differentiated, not enclosed	Energ/Metab		Putative adenylosuccinate lyase					DM	SC	hs	granular cytoplasm
T01G9.4			Differentiated, not enclosed	Unknown	*kup-2*				x		DM		hs	P1 divides too late
Cytoplasmic Appearance														
F26H9.6			200-500 cells	Cell Bio/Arch	*rab-5*	ras superfamily GTPase				CE	DM	SC	HS	clear
F57B10.1			Differentiated, not enclosed	Specific Txn		bZIP transcription factor					dm		hs	clear
F18C12.2			Enclosed	Unknown		DnaJ domain				CE			hs	clear

Phenotypes arising from RNAi of 147 embryonic lethal genes on chromosome I. Genes are grouped by phenotypic class. The following data are shown: the Research Genetics GenePair name; whether the sequence might target two or more unrelated genes [shaded box; determined in Fraser *et al.* (2000)]; whether the GenePair appears to target the same gene as another GenePair (shaded box); whether the sequence amplified by the GenePair is >80% identical to more than one predicted gene such that it might target two or more paralogous genes [shaded box; determined in Fraser *et al.* (2000)]; the terminal phenotype; the functional class [as defined in Fraser *et al.* (2000)]; the corresponding genetic locus name if it exists; a description of the gene sequence [as defined in Fraser *et al.* (2000)]; three columns showing whether the gene was found to have oocyte-specific (Ooc), sperm-specific (Sperm) or germline-intrinsic (GL intr) expression in Reinke *et al.* (2000); existence of matches (lower case) or homologues (filled box, white upper-case text) in *C.elegans* (CE), *Drosophila melanogaster* (DM), *Saccharomyces cerevisiae* (SC) or humans (HS) [as determined in Fraser *et al.* (2000)]; and additional phenotypic information. Phenotypic classes are defined as follows: '1-Cell Arrest', embryos arrest as a single cell, usually without attempting mitosis; 'Meiosis', embryos have multiple or no female pronuclei; 'Pronuclear Envelope', the maternal and paternal pronuclear envelopes appear indistinct; 'Pronuclear Migration', pronuclei do not meet due either to complete or partial failure to migrate; 'Spindle Assembly', the mitotic spindle is absent or appears abnormal; 'Spindle Orientation', the orientation of division of at least one cell in the first three divisions is incorrect; 'Spindle position', correctly oriented spindles are mispositioned within cells, including spindles that are central, too posterior, skewed, or unstable; 'Spindle Orientation/Position', embryos display features of both classes; 'Cytokinesis', cytokinetic furrows fail to form, are

TCF homologue that acts in the Wnt pathway (Lin *et al*., 1995), as well as genes not previously investigated, such as a homologue of *Drosophila eyelid*. *eyelid* encodes a DNA-binding domain protein of the BRIGHT family and appears to be antagonistic to wingless signalling (Treisman *et al*., 1997).

Other																		
C47B2.4				1-12 cell arrest	Prot Syn/Deg		proteasome subunit					ce	DM	SC	HS	very active cytoplasm; huge polar bodies
F32H2.6 (b)				<200 cells	Energ/Metab		fatty acid synthase (N-terminus)					CE	DM			polar bodies absorbed during first cleavage
F32H2.5				200-500 cells	Energ/Metab		fatty acid synthase					ce	dm	sc	hs	polar bodies absorbed during first cleavage
K07A1.12				<200 cells	RNA Syn/Proc	*rba-2*	human RbAp46/48 homologue				x	CE	DM	SC	hs	abnormal nuclear morphology
																only late embryonic phenotype
R09B3.4				<200 cells	Prot Syn/Deg		ubiquitin-conjugating					ce	dm	sc	hs	no pronuclear migration
																furrow regresses
																posterior first spindle
M01A10.3				<200 cells	Cell Bio/Arch		2 genes: ribophorin /lethal giant larvae homologue									multiple female pronuclei
																spindle rotation in AB
F57B10.10				<200 cells	Unknown	*dad-1*							DM	sc		multiple female pronuclei
																spindle rotation in AB
Y71F9A_279.b				200-500 cells	Cell Bio/Arch		NTF2-related NXT1 orthologue						dm	sc		multiple female pronuclei
																additional aster-like material
Late Embryonic Phenotype																		
C32E8.8				<200 cells	Energ/Metab		homologous to Niemann-Pick type C	x				CE	DM	sc	hs	
C36B1.3				<200 cells	RNA Syn/Proc		RNA pol II subunit				x	ce	dm	SC	hs	
C50F2.3				<200 cells	RNA Syn/Proc		putative splicing factor	x				ce	DM	SC	hs	
D1081.8				<200 cells	RNA Syn/Proc		Myb-like; role in splicing				x		DM	sc	hs	
F31C3.5				<200 cells	Unknown								DM	SC		
F43G9.10				<200 cells	Cell Bio/Arch		Microfibrillar AP homologue				x		dm		hs	
F55A3.3				<200 cells	RNA Syn/Proc		general chromatin factor; Spt16p homologue					ce	DM	SC	HS	
F55F8.4				<200 cells	Specific Txn		sim to hCIR; transcriptional repression						dm			
H25P06.2a				<200 cells	RNA Syn/Proc		CDK9 orthologue					CE	DM	SC	hs	
K02F2.3				<200 cells	RNA Syn/Proc		splicing factor; U2 snRNP ass. protein					CE	DM	SC	hs	
Y48G10A.b				<200 cells	Unknown		coiled coils									
Y54E10B_159.c				<200 cells	RNA Syn/Proc		RNA pol II subunit						DM	SC	HS	
Y65B4B_10.c				<200 cells	Unknown											slow
C36B1.5				200-500 cells	RNA Syn/Proc		splicing factor; U2/4 snRNP ass. protein				x	ce	DM	sc	hs	
F30F8.8				200-500 cells	RNA Syn/Proc		TAF; WD repeat				x	ce	DM	sc	hs	
F36F2.3				200-500 cells	RNA Syn/Proc		C-t like RNApol II CTD phosphatase						dm	sc	hs	
F39H11.2				200-500 cells	RNA Syn/Proc		TLF (TB- like factor)					ce	dm	sc	hs	
F49D11.1				200-500 cells	RNA Syn/Proc		putative splicing factor; WD-repeat					CE	DM	SC	HS	
Y110A7A.m				200-500 cells	RNA Syn/Proc		splicing component; yeast PRP31 homologue					ce	DM	sc	hs	
C06A5.1				Differentiated, not enclosed	Unknown								dm		hs	
F33D11.10				Differentiated, not enclosed	Prot Syn/Deg		translational initiation factor/helicase	x				CE	DM	SC	HS	
F43G9.12				Differentiated, not enclosed	Specific Txn		Hu TCF-9-like								hs	
F46A9.5				Differentiated, not enclosed	Prot Syn/Deg		2 genes: both SKP1 family members									
F53G12.5				Differentiated, not enclosed	RNA Syn/Proc	*mex-3*	KH domain						dm		hs	cytokinesis defects seen in rare cases
K07A1.11				Differentiated, not enclosed	RNA Syn/Proc	*rba-1*	human RbAp46/48 homologue					CE	DM	SC	hs	
T23D8.4 (c)				Differentiated, not enclosed	Signalling		weak *wrm-1* homologue									
T23D8.9				Differentiated, not enclosed	Signalling		weak *wrm-1* homologue									
W03D8.4				Differentiated, not enclosed	Specific Txn	*pop-1*	HMG box protein						dm		hs	
W04A4.6				Differentiated, not enclosed	Unknown								dm			
Y54E10A_156.a				Differentiated, not enclosed	Unknown											
Y54E10B_152.e				Differentiated, not enclosed	-		2 genes: MAPKK /similar to collagen									
Y54E5B.3				Differentiated, not enclosed	Specific Txn		Mediator complex subunit						dm	sc		
Y71A12B.a				Differentiated, not enclosed	RNA Syn/Proc		sim to TAF2A component of TFIID						DM	sc	HS	
Y71A12B.b (a)				Differentiated, not enclosed	Unknown								DM	sc	HS	
C32F10.5 (a)				Enclosed	Specific Txn		SSRP1 homolog				x	CE	dm	sc	hs	
C53D5.a				Enclosed	Cell Bio/Arch		karyopherin-beta homologue					ce	DM	SC	HS	osmotically sensitive eggs
C53D5.i				Enclosed	Cell Bio/Arch		karyopherin-beta homologue					ce	DM	SC	HS	osmotically sensitive eggs
K12C11.2				Enclosed	Signalling		SUMO-1 like						DM	SC	HS	
M05B5.5				Enclosed	Specific Txn	*hlh-2*	bHLH transcription factor									
Y23H5A.3				Enclosed	Unknown						x					
Y47G6A_247.i				Enclosed	Cell Cyc/Chrm		S pombe Rad2 homologue					ce	DM	SC	HS	
B0414.1				1-2fold	Unknown							CE				
C01G8.7				1-2fold	Specific Txn		eyelid-like						dm			
C01G8.8				1-2fold	Specific Txn		eyelid-like						dm			
C10H11.9				1-2fold	Signalling	*let-502*	ROCK					ce	DM	sc	hs	
E01A2.f				1-2fold	Unknown		coiled coils									
F53B8.1				1-2fold	Unknown		kakapo homologue					CE	DM	sc	hs	
K05C4.6				1-2fold	Signalling	*hmp-2*	beta-catenin homologue	x								
R09B3.5				1-2fold	Unknown		mago nashi functional orthologue						DM		hs	
Y105E8C.d				1-2fold	Unknown								dm	sc		
Y105E8C.n				1-2fold	Cell Bio/Arch		gamma-adaptin AP-1					ce	dm	sc	hs	
F27D4.2				>2-fold	Unknown		coiled coils									
Slow Development																		
C17E4.5					RNA Syn/Proc		poly-A binding protein	x				ce	dm	sc	hs	
C34B2.8					Unknown								DM		HS	
C54G4.8					Energ/Metab		cytochrome c1						DM	SC		
F36A2.7					Unknown							CE				
F37F2.1					Energ/Metab		mitochondrial ribosomal protein L15						DM			
Variable																		
C09D4.5				Variable arrest	Prot Syn/Deg		ribosomal protein L19						DM	SC	hs	multiple nuclei
F25H2.4				Variable arrest	Unknown								DM		HS	slow
F37E3.1				Variable arrest	RNA Syn/Proc		mRNA CAP binding						DM	sc	hs	
F39B2.6				Variable arrest	Prot Syn/Deg		ribosomal protein S26						dm	sc	hs	slow
F43G9.1				Variable arrest	Energ/Metab		NAD+-isocitrate dehydrogenase					CE	DM	SC	HS	slow
F46F11.5				Variable arrest	Cell Bio/Arch		vacuolar ATPase						DM	SC	HS	
F52B5.6				Variable arrest	Prot Syn/Deg		ribosomal protein L25					CE	DM	SC	HS	slow; multiple nuclei
H28O16.1				Variable arrest	Energ/Metab		ATP synthase					ce	DM	SC	HS	early arrest
K07A12.3				Variable arrest	-		2 genes: ATP synthase / Transl. Init. Factor									slow
T08B2.9				Variable arrest	Prot Syn/Deg		tRNA synthetase						DM	SC	HS	slow; extra female pronuclei
T09B4.9				Variable arrest	Energ/Metab		mitoch. inner membrane translocase						DM	SC	hs	slow; patchy cytoplasm
T10E9.7				Variable arrest	Energ/Metab		NADH oxidoreductase						dm			slow
Y105E8C.e				Variable arrest	Prot Syn/Deg		ribosomal protein S20						dm	sc	hs	
ZC434.2				Variable arrest	Prot Syn/Deg		ribosomal protein S7						DM	SC	HS	multiple nuclei

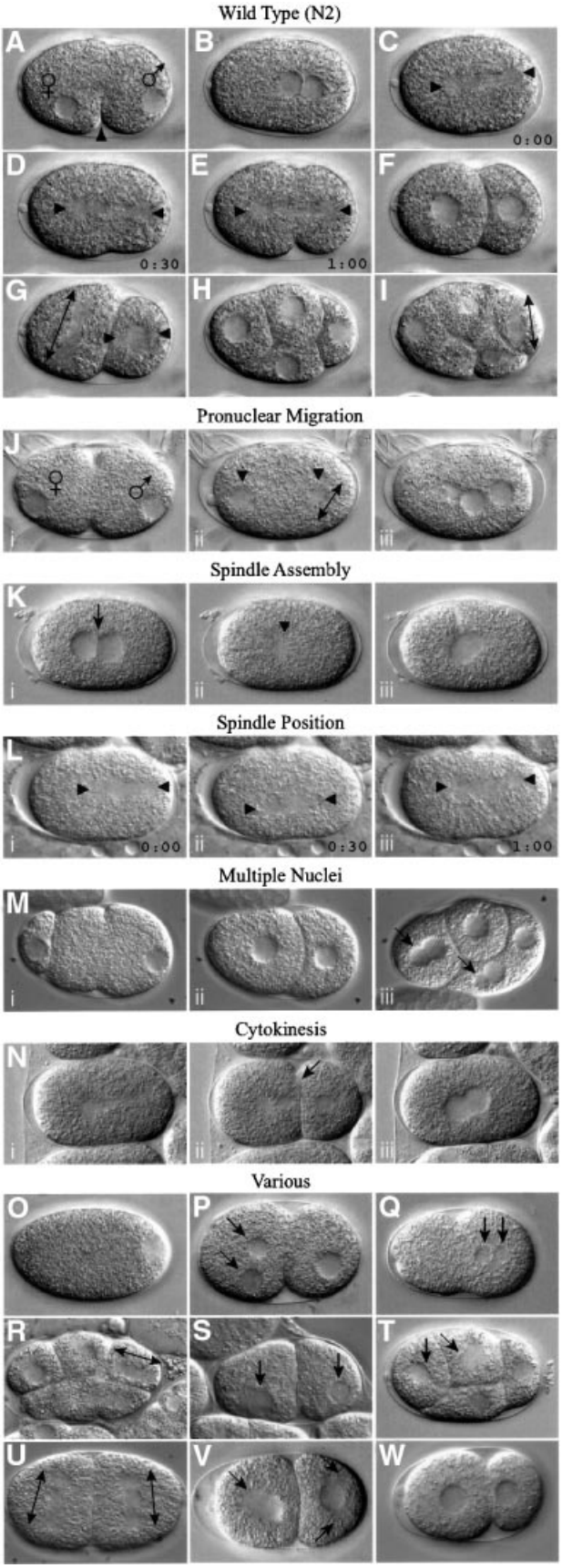
incomplete, or occur ectopically; 'Multiple Nuclei', cells contain more than one nucleus, but meiosis is apparently normal as a single maternal pronucleus is present (probable chromosome segregation defect); 'Nuclear Position', nuclear position is unstable or abnormal; 'Nuclear Morphology', nuclei are either abnormally shaped or fail to reform after division, but pronuclei appear normal (the defect is usually first seen strongly in ABa and ABp); 'Cytoplasmic Appearance', cytoplasm appears clear and less granular than wild type; 'Other', genes where different recorded embryos showed different phenotypes or genes where a phenotype could not be assigned to a particular phenotypic class; 'Late Embryonic Phenotype', normal early embryonic development and a consistent terminal arrest phenotype; 'Slow Development', normal, but slow development; 'Variable', variable terminal arrest phenotype (early development is often slow).

[a]Note that genes C32F10.5, F55A3.7 and Y71A12B.b were found to be embryonic lethal subsequent to publication of Fraser *et al*. (2000).

[b]F32H2.6 shows 78% identity over 320 bp to F32H2.5.

[c]GenePair T23D8.4 does not target predicted gene T24D8.4, but the neighbouring predicted gene T24D8.9.

Another trend is that LEP >500 cell genes have a higher proportion of genes of unknown function than those with Early Embryonic Phenotypes (EEP; 34% versus 20%). This may reflect the fact that the basic processes of eukaryotic cell biology have been well studied in comparison to later developmental events. A high proportion (50%) of these genes of unknown function have homologues in other organisms (Table I) and are therefore likely to have conserved functions. Overall, the embryonic lethal genes analysed here are highly conserved: 84% have a match in humans, *Drosophila* or yeast (Table I), in comparison with 74% for all genes with an RNAi phenotype (Fraser *et al*., 2000).



Comparison with germline expression data

Recently, microarray experiments identified *C.elegans* oocyte-enriched, sperm-enriched and germline-intrinsic (germline expression not specific to sperm or oocytes) genes (Reinke *et al*., 2000). We examined the distribution of chromosome I phenotypes among these genes. The frequency of embryonic lethal RNAi phenotypes among the germline-intrinsic and oocyte-enriched genes is much higher than for genes on chromosome I in general (27 and

Fig. 1. Wild-type and RNAi mutant embryos. (A–I) Development of a wild-type embryo from the one- to the eight-cell stage. After fertilization, the oocyte nucleus completes meiosis and two polar bodies are extruded (not shown). (A) Subsequently the female and male pronuclei become visible. (**A** and **B**) The single oocyte pronucleus migrates to meet the sperm pronucleus in the posterior of the one cell embryo; this is accompanied by a pseudocleavage furrow [arrowhead in (A)]. (**C**–**E**) The first mitotic spindle is set up, elongating in the posterior direction through a rocking movement (arrowheads show ends of the spindle). (**F**) Two-cell embryo; first cleavage results in a larger anterior cell (AB) and a smaller posterior cell (P1). (**G**) The anterior AB cell divides before P1, along the short axis of the egg (AB spindle is shown by a double-headed arrow); P1 will divide along the anterior–posterior axis due to nucleocentrosomal rotation, which places centrosomes along this axis (arrowheads). (**H**) Four-cell embryo. (**I**) Division of P2 cell; the double-headed arrow shows orientation of the spindle. (J–N) Three time points from video recordings of each of five different RNAi mutants. (**J**) Pronuclear Migration; T06G6.9. Pronuclei fail to migrate. The embryo in (Jii) is similar stage to (C); pronuclear envelopes have broken down and sperm pronucleus has set up a small spindle (double-headed arrow). (**K**) Spindle Assembly; F32H2.3. (Kii) The mitotic spindle fails to set up [the arrowhead marks a small clear area where pronuclei have broken down; compare to spindle in (D)]. Note that the pronuclei meet centrally [arrow in (Ki)]. (**L**) Spindle Position; F21C3.5. The position of the mitotic spindle (marked by arrowheads) is unstable. Compare (Li–Liii) to (C–E). Individual images have been taken at 30 s intervals. (**M**) Multiple Nuclei; F56C11.5. Two cells each have two nuclei after division [arrows in (Miii)]. (**N**) Cytokinesis; Y18D10A.17. A normal cytokinesis furrow [arrow in (Nii)] regresses (Niii). (O–W) Single pictures illustrating other phenotypes. (**O**) 1-Cell Arrest; R12E2.3. (**P**) Meiosis; M01E11.6. Multiple female pronuclei are visible (arrows). (**Q**) Pronuclear Envelope; F56A3.3. The pronuclei have an indistinct appearance in comparison to (B). (**R**) Spindle Orientation; Y18D10A.5. Embryos have an abnormal P2 spindle orientation [compare to (I)]. (**S**) Nuclear Position; Y65B4B_10.b. Nuclei (arrows) are not centrally located in comparison with (F). (**T**) Nuclear Morphology; F33H2.5. Nuclei (arrows) are irregularly shaped and somewhat indistinct in comparison to the clear round nuclei in (H). (**U**) Spindle Orientation/ Position; F20G4.3. AB and P1 cells divide at the same time, and both spindles exhibit a transverse orientation [compare to (G)]. The identical size of the two cells is reflective of a centrally located P0 spindle. (**V**) Aster Morphology (from Multiple Nuclei class); F26E4.1. Asters are very large and can easily be seen as clear regions next to the nuclei (marked by arrows). (**W**) Cytoplasmic Appearance; F26H9.6. The cytoplasm appears clear and less granular than wild type (F). Table I gives further information about the examples shown here. In all images, posterior is to the right.

A Distribution of Genes among Phenotypic and Functional Classes

Phenotypic Class	DNA Syn	RNA Syn/Proc	Prot Syn/Deg	Energ/Metab	Cell Cyc/Chrm	Cell Bio/Arch	Signalling	Specific Txn	Unknown	Multiple	total	Expected in Genome
1-Cell Arrest			7		1				1	1	10	108
Meiosis					4	1					5	54
Pronuclear Envelope						7			1		8	86
Pronuclear Migration			1			1					2	22
Spindle Assembly			1			2			4		7	76
Spindle Orientation			1			1	3		1		6	65
Spindle Position						1			2	1	4	43
Spindle Position/Orientation						2			1		3	32
Cytokinesis		1		1	1	2					5	54
Mutiple Nuclei					1	1	1		1		4	43
Nuclear Position			2		1						3	32
Nuclear Morphology	3	2		1					2		8	86
Cytoplasmic Appearance						1		1	1		3	32
Other		1	2	2		2			1		8	86
EEP total	3	4	14	4	8	21	4	1	15	2	76	821
%	4	5	18	5	11	28	5	1	20	3	100	
LEP <500 Cells		13		1		1		1	3		19	205
%	0	69	0	5	0	5	0	5	16	-	100	
LEP >500 Cells		3	2		1	3	5	7	11	1	33	356
%	0	9	6	0	3	9	15	21	34	3	100	
LEP total		16	2	1	1	4	5	8	14	1	52	561
%	0	31	4	2	2	8	10	16	27	-	100	
Slow Development		1		2					2		5	86
Variable		1	6	4		1			1	1	14	270

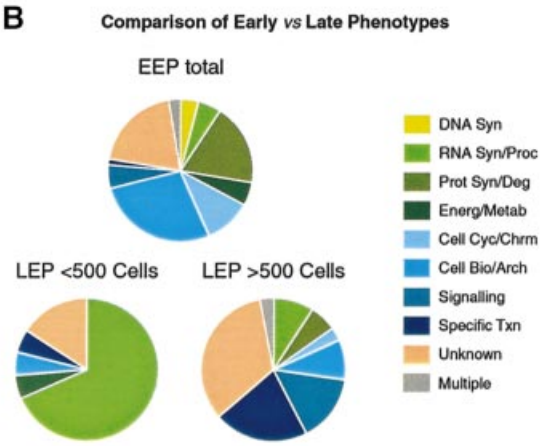


Fig. 2. Distribution of genes among phenotypic and functional classes. (**A**) The first column gives phenotypic classes. EEP, early embryonic phenotype; LEP, late embryonic phenotype. Each row gives the number of genes with the given RNAi phenotype in each functional class, the total number in that class identified in this screen, and the total number in that class expected to be found in the genome by RNAi (see Materials and methods for the latter calculation). (**B**) Pie charts showing the distribution of functional classes among genes within the EEP, LEP <500 and LEP >500 phenotypic groupings. Functional classes were used as defined in Fraser *et al*. (2000).

18%, respectively, versus 9%; Fraser *et al*., 2000; $p < 0.05$), consistent with their germline expression. Interestingly, only one of 128 sperm-enriched genes on chromosome I had an RNAi phenotype (Fraser *et al*., 2000). The reason for this may be that genes involved in spermatogenesis appear resistant to RNAi or that our screening procedure was not suitable for identifying these genes. The lack of any other RNAi phenotype in almost all genes in this class is consistent with the proposal that these genes have sperm-specific functions; the one sperm-enriched gene that did give an RNAi phenotype produced sterility in the progeny, although we do not yet know whether this is the result of defective sperm (Fraser *et al*., 2000).

Genes with germline-intrinsic expression are found in most phenotypic classes, but show two clusterings. First, half of the genes in the Nuclear Morphology phenotypic class are germline intrinsic. Genes in this class are frequently involved in chromatin regulation or DNA replication. These genes would be expected to be germline expressed since this is the only tissue actively undergoing nuclear division in the adult. Secondly, in the LEP class, germline-intrinsic genes are clustered in the LEP <500 cell class, which contains many proteins involved in basal transcription. That so many of such genes are expressed at high levels in the germline can perhaps be explained by the fact that germline cytoplasm is rapidly turning over as oocytes are made. Phenotypic defects in the embryo indicate that these proteins may act both in the germline and the embryo.

Discussion

We have taken a systematic approach to identify genes required for early embryonic processes such as mitosis, the regulation of spindle position, cytokinesis, and the establishment of polarity. In this study, we present phenotypic information on 147 embryonic lethal genes that were initially identified in a genome-wide RNAi screen of *C.elegans* chromosome I (Fraser *et al*., 2000). Over half of the genes analysed have a detectable defect in the first three cell divisions. For these and for the genes without an early defect, the terminal arrest phenotypes provide further information on gene function. This work provides a starting point for further studies of cell division and other processes.

One striking finding from our studies is that some phenotypic classes primarily contain genes involved in a shared biochemical process. For example, many genes whose RNAi resulted in one-cell arrest encode proteasome or APC components, those that cause altered pronuclear envelope morphology are often involved in nuclear transport or are components of the nuclear envelope, and those resulting in abnormal nuclear morphology are often involved in the packaging or synthesis of DNA. Such correlation between phenotype and cellular function can

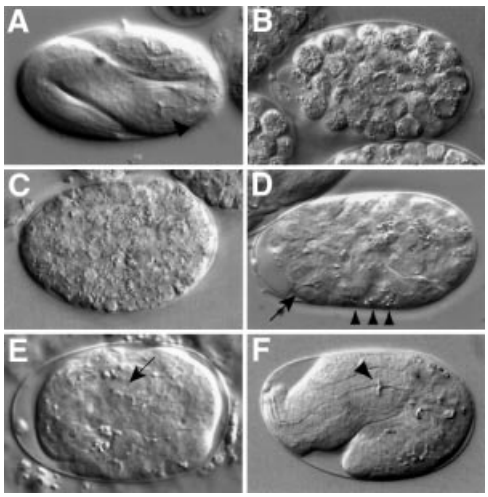


Fig. 3. Terminal phenotype classes. (**A**) Wild-type embryo at 3-fold stage of elongation. The pharyngeal grinder is marked with an arrowhead. (**B**) <200 cell arrest; K02F2.3. (**C**) 200–500 cell arrest; F30F8.8. (**D**) Differentiated, not enclosed; T23D8.4. The embryo is not enclosed within epidermal tissue [compare with (E)] such that pharyngeal tissue (arrow) and some gut cells (arrowheads) are found at the surface of the embryo. (**E**) Enclosed; C32F10.5. Pharynx (arrow) is surrounded by epidermal tissue. (**F**) 1- to 2-fold arrest; F53B8.1. Pharynx is properly formed with a normal grinder (arrowhead), but the embryo is not properly elongated [compare with (A)].

be used to inform analyses of genes whose biochemical function is unknown. Genes of unknown biochemical function occur in the phenotypic classes above, suggesting that these genes might encode new factors involved in the identified processes.

A similar RNAi study of genes on *C.elegans* chromosome III identified early embryonic phenotypes for 133 genes after video recording the first two cell divisions (Gönczy *et al*., 2000). The range of early phenotypes seen in that study is comparable to those observed here. However, two major differences in approach have allowed us to provide phenotypic information for a higher fraction of embryonic lethals analysed, and to carry out the analyses more rapidly. First, in addition to looking for defects during early cleavages, we also characterized genes with no early RNAi phenotype (nearly half of embryonic lethal genes) by examining terminal arrest phenotypes. This analysis identified a common arrest phenotype after RNAi of genes involved in basal transcription, and further identified candidate genes for late embryonic processes such as embryonic elongation. Secondly, we first determined which genes had an embryonic lethal RNAi phenotype using a rapid RNAi by feeding approach (Fraser *et al*., 2000) and then analysed those by video microscopy. In contrast, Gönczy *et al*. (2000) collected video recordings after RNAi of nearly all genes on chromosome III (2174 genes); an early embryonic phenotype was seen for only 6% of them. As 94% of chromosome III genes with an early embryonic RNAi phenotype were found to be embryonic lethal (Gönczy *et al*., 2000), our pre-screening step should not have caused us to miss many relevant genes. The video recording step is the most labour-intensive part of the process; thus, the pre-screening step reduces the effort required significantly, as <10% of genes give an embryonic lethal RNAi phenotype (Fraser *et al*., 2000; Gönczy *et al*., 2000).

RNAi of *C.elegans* ovary cDNAs was also recently used to identify genes involved in early development (Piano *et al*., 2000). The cDNA approach has the benefit of enriching for genes with an early role in development (~30% of the genes analysed had an embryonic lethal RNAi phenotype, compared with 9% for chromosome I). However, the genes identified will be skewed towards those with high expression. In addition, a comparison with our chromosome I-wide results suggests that some classes of phenotypes are overrepresented in the cDNA approach. For example, Piano *et al*. (2000) found that nearly half of the genes with a detectable early RNAi phenotype had a one-cell arrest phenotype and were involved in protein synthesis or turnover, whereas this class represented only 5% of the genes in our study. A genome-wide screen has the advantage that nearly every gene can be assayed, irrespective of expression level.

The efficiency of our screen can be estimated by comparison of the phenotypes we obtained to those previously reported for genes on chromosome I (Table II). Of the 25 sequenced genes on chromosome I previously shown to give an embryonic lethal phenotype and also analysed in Fraser *et al*. (2000), we found that 20 (80%) were embryonic lethal by RNAi (Fraser *et al*., 2000); this includes all of the seven previously shown to have defects in the first three cell divisions (*dhc-1*, *icp-1*, *mei-1*, *mei-2*, *mel-26*, *nmy-2* and *par*-6). Of these 20, we recorded 17 in this study; in each case, we obtained the published phenotype (Table II). Therefore, our approach should be able to identify specific RNAi phenotypes for ~80% of the non-redundant genes required for embryonic development.

Scaling this approach up to the entire genome, we estimate that 821 genes with an early RNAi phenotype and 561 with a late one could be identified by RNAi (see Materials and methods for calculation). Figure 2 gives individual numbers for each phenotypic class; for example, RNAi might identify 54 genes involved in meiosis. Moreover, of the 821 early genes that could be identified by RNAi, ~162 of these are expected to be of unknown biochemical function, and half will have homologues in other organisms. This work will therefore provide insights into the functions of these novel genes. When complete, an analysis of early RNAi phenotypes should identify the majority of genes involved in a wide range of cell processes and thus allow rapid progress to be made in understanding their molecular mechanisms.

Materials and methods

dsRNA generation

RNAi was accomplished either by injection or by feeding as in Kamath *et al*. (2000). Supplementary table I (Supplementary data are available at *The EMBO Journal* Online) shows the number of recordings made for each gene, which method was used, and in which a mutant phenotype was observed. Overall, the classified phenotype was observed by injection and feeding in 76% of the cases (n = 93). Twenty-one per cent showed the classified phenotype by injection only, and in 3% a phenotype was seen by feeding only. For injections, dsRNA for each gene was generated as follows: PCR using 25 pmol of T7 oligo (5′-CGTAATACGACTCACTATAG-3′; 95°C for 30 s, 52°C for 30 s, 72°C for 90 s, 25 cycles) was performed on bacteria containing the gene-specific fragment of interest cloned into the L4440 vector (Timmons and Fire, 1998; Fraser *et al*., 2000). Inserts in this vector are flanked by inverted T7 RNA polymerase promoters; hence, *in vitro* transcription of these PCR fragments using T7 polymerase generates sense and antisense strands in

Table II. Comparison of reported embryonic phenotypes with phenotypes presented in this study

Locus	GenePair	Description	Mutation/ RNAi	Reported Phenotype	% *emb* by Feeding	Phenotypic Class	Term. Phenotype Class	similar	Ref.
dhc-1	T21E12.4	dynein heavy chain	RNAi	Pronuclear Migration	100	Pronuclear Migr.	<200 cells	✓	a
ego-1	F26A3.3	RNA-directed RNA pol.	mut	20-50 cell arrest	50-80	Cytokinesis	<200 cells	✓	b
gsk-3	Y18D10A.5	GSK-3beta homologue	RNAi	Spindle Orientation	100	Spindle Orientation	Enclosed	✓	c
hlh-2	M05B5.5	bHLH transcription factor	RNAi	emb, morph. defects	100	LEP	Enclosed	✓	d
hmp-2	K05C4.6	beta-catenin homologue	mut	1.5 fold Arrest	100	Spindle Orientation	1-2 fold arrest	✓	e
icp-1	Y39G10A_246.i	INCENP-like	RNAi	Cytokinesis	100	Cytokinesis	1-12 cell arrest	✓	f
let-502	C10H11.9	ROCK	mut	1.5 fold Arrest	50-80	LEP	1-2 fold arrest	✓	g
mei-1	T01G9.5	AAA ATPase	mut	Meiosis	100	Meiosis	Diff.; not encl.	✓	h
mei-2	F57B10.12	katanin homologue	mut/RNAi	Meiosis	100	Meiosis	Diff.; not encl.	✓	i
mel-26	ZK858n4	BTB domain	mut	Spindle Position	100	Spindle Position	Diff.; not encl.	✓	j
mex-3	F53G12.5	KH domain	mut	Diff.; not encl.	100	LEP	Diff.; not encl.	✓	k
nmy-2	F20G4.3	non-muscle myosin	RNAi	Spindle Pos./Orient.	50-80	Spindle Pos./Orient.	Diff.; not encl.	✓	l
par-6	T26E3.3	PDZ domain	mut	Spindle Pos./Orient.	100	Spindle Pos./Orient.	Diff.; not encl.	✓	m
pop-1	W03D8.4	HMG box protein	mut	Diff.; not encl.	100	LEP	Diff.; not encl.	✓	n
rba-1	K07A1.11	human RbAp46/48 hom.	RNAi	Diff.; not encl.	100	LEP	Diff.; not encl.	✓	o
rba-2	K07A1.12	human RbAp46/48 hom.	RNAi	Diff.; not encl.	100	LEP (*)	Diff.; not encl.	✓	o
apr-1	K04G2.8	APC	mut	Diff.; not encl.	none	-	-	NA	p
chp-1	Y110A7A.g	CHORD-domain prot.	RNAi	Emb, red. fecundity	100	Spindle Pos./Orient.	Diff.; not encl.	NA	q
hmr-1	W02B9.1	cadherin	mut	Diff.; not encl.	10	-	-	NA	e
mom-4	F52F12.3	Kinase, TAK-1 homolog	mut	Diff.; not encl.	none	-	-	NA	r
mom-5	T23D8.1	Frizzled	mut	Diff.; not encl.	none	-	-	NA	p, r
nhr-2	C32F10.7	nuclear hormone receptor	RNAi	Diff.; not encl.	none	-	-	NA	s
sup-17	DY3.7	ADAM metalloprotease	mut	Emb, Dpy, Unc	none	-	-	NA	t
unc-37	W02D3.9	WD repeat	mut	Unc, Emb, Ste	100	-	-	NA	u
unc-73	F55C7.4	GEF	mut	Unc, Emb	20-40	-	-	NA	v

a, Gönczy *et al*., 1999; b, Smardon *et al*., 2000; c, Schlesinger *et al*., 1999; d, Krause *et al*., 1997; e, Costa *et al*., 1998; f, Kaitna *et al*., 2000; g, Wissmann *et al*., 1997; h, Clandinin and Mains, 1993; i, Srayko *et al*., 2000; j, Dow and Mains, 1998; k, Draper *et al*., 1996; l, Guo and Kemphues, 1996; m, Watts *et al*., 1996; n, Lin *et al*., 1995; o, Shi and Mello, 1998; p, Rocheleau *et al*., 1997, q, Shirasu *et al*., 1999; r, Thorpe *et al*., 1997; s, Sluder *et al*., 1997; t, Tax *et al*., 1997; u, Pflugrad *et al*., 1997; v, Steven *et al*., 1998.

Comparison of phenotypes of previously known loci to those identified in this study. Shown are the locus, the GenePair covering that locus, the gene description, whether the gene was previously studied using a mutant (mut) or RNAi, the reported phenotype, whether it was identified as embryonic lethal (Emb) in Fraser *et al*. (2000), our phenotypic class, our terminal phenotype class, whether our phenotype matches the published phenotype, and reference for the published phenotype. The upper part of the table lists the genes in this study and hence those that could be compared with published phenotypes; the lower part lists the genes without comparison either because we obtained no or low embryonic lethality or maternal sterility (Ste), or because the published phenotype was not detailed enough. A tick indicates our phenotypic class or terminal class, or both are similar to published. We could not compare the *chp-1* phenotypes as no specific phenotype (besides embryonic lethality and reduced fecundity) was reported. *For K07A1.12, our two analyses yielded two different phenotypic classes: Late Embryonic Phenotype/Differentiated, not enclosed in one case and Nuclear Morphology/<200 cell arrest in the other case. Note that *air-2* is missing from this list because it was not analysed in Fraser *et al*. (2000). LEP, late embryonic phenotype; NA, not applicable.

the same transcription reaction. PCR products were used directly as templates in transcription reactions (4.5 h at 37°C, 10 min at 72°C, RiboMAX™ RNA Production kit from Promega). dsRNA was analysed on a gel for verification of its size and concentration. Transcription reactions containing dsRNAs were diluted with 10 mM Tris–HCl pH 7.2, 0.1 mM EDTA about five times to a concentration of 0.5–1.0 mg/ml for injection. dsRNA was injected into the distal arms of both gonads of young hermaphrodites. Injected worms were kept at 22°C for 24 h prior to dissection of embryos. When feeding was used, L4 hermaphrodites were placed on induced feeding bacteria and left for 3 days at 15°C before dissection of embryos.

Video recordings

After RNAi, hermaphrodites were placed on a coverslip in egg buffer (118 mM NaCl, 40 mM KCl, 3 mM $CaCl_2$, 3 mM $MgCl_2$, 5 mM HEPES pH 7.2), cut open to release embryos, and then the coverslip was mounted onto a 3% agar pad and sealed with petroleum jelly. In general, the development of at least two embryos (from two different mothers) was video recorded per GenePair using Improvision OpenLab™ software. In some cases in the 1-Cell Arrest and Variable classes, progeny of only one mother were analysed. Four-dimensional time-lapse video recordings consisting of 12 optical sections were taken every 30 s for 100 time points, usually beginning before pronuclear migration and ending after the third round of divisions. From each recording, a single focal plane movie consisting of a middle focal plane was made for analysis and for deposition into the *C.elegans* database WormBase (http://www.wormbase.org). After completion of recordings, slides were kept at 15°C for 24 h prior to collecting a single series of 24 focal planes for analysis of the terminal phenotype.

Data analyses

Names given in all figures and tables are GenePair names and not names of predicted genes. GenePair names do not always match current gene names as gene predictions can change as they are improved, but GenePair names will always match the phenotype identified. The current matching of GenePair to gene can be found in WormBase (www.wormbase.org). About 95% of GenePairs have a one-to-one match with a currently predicted gene. Of the 226 genes found previously to have an embryonic lethal RNAi phenotype, 131 gave >50% embryonic lethality with no maternal sterility. We analysed 113 (86%) of these. In addition, we were able to analyse embryos after RNAi of 34 of 73 (46%) genes where RNAi induced partial or complete sterility in the mother.

The number of genes expected to be found by RNAi was calculated as follows. We identified 226 embryonic lethal genes after screening 13% of the genome (Fraser *et al*., 2000). We completed assays on 147 here; we identified a further three in the Slow Development and 11 in the Variable classes for which we only have one observation. These latter genes were included in the calculation for the expected number of genes in the Slow Development and Variable classes in Figure 2. Therefore, for each phenotypic class, RNAi could identify [(number of genes in class) × 226]/(147 + 11 + 3)/0.13 genes in the entire genome.

Supplementary data

Supplementary data for this paper are available at *The EMBO Journal* Online.

Acknowledgements

We are grateful to R.Durbin and M.Gotta for helpful comments on the manuscript. P.Z. was supported by a Wellcome Trust Studentship and is a

Junior Research Fellow at Hughes Hall College, A.G.F. by a US Army Breast Cancer Research Fellowship, R.S.K. by a Howard Hughes Medical Institute Predoctoral Fellowship, M.M.-C. by an EC-TMR Network Grant, and J.A. by a Wellcome Trust Senior Research Fellowship (054523).

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Received March 28, 2001; revised June 13, 2001; accepted June 19, 2001