12 Le Beau, M.M. *et al.* (1998) *Hum. Mol. Genet.* 7, 755–761
13 Sbrana, I., Zavattari, P., Barale, R. and Musio, A. (1998) *Hum. Genet.* 102, 409–414
14 Huang, H., Qian, C., Jenkins, R.B. and Smith, D.I. (1998) *Genes Chromosomes Cancer* 21, 152–159
15 Yu, A., Bailey, A.D. and Wiener, A.M. (1998) *Hum. Mol. Genet.* 7, 609–617
16 Li, Z., Yu, A. and Wiener, A.M. (1998) *J. Virol.* 72, 4183–4191
17 Malter, H.E. *et al.* (1997) *Nat. Genet.* 15, 165–169
18 Bakker, C.E. *et al.* (1994) *Cell* 78, 23–33
19 Nancarrow, J.K. *et al.* (1994) *Science* 264, 1938–1941
20 Chiurazzi, P. *et al.* (1998) *Hum. Mol. Genet.* 7, 109–113
21 Gecz, J., Gedeon, A.K., Sutherland, G.R. and Mulley, J.C. (1996) *Nat. Genet.* 13, 105–108
22 Chakrabarti, L., Knight, S.J.L., Flannery, A.V. and Davies, K.E. (1996) *Genomics* 44, 201–203
23 Gu, Y., Shen, Y., Gibbs, R.A. and Nelson, D.L. (1996) *Nat. Genet.* 13, 109–113
24 Gecz, J. *et al.* (1997) *Genomics* 44, 201–203
25 Teague, J.W. *et al.* (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 719–724
26 Mornet, E., Chateau, C., Simon-Bouy, B. and Serre, J.L. (1998) *Clin. Genet.* 53, 200–201
27 Chan, S.Y. and Wong, V. (1998) *Clin. Genet.* 53, 179–183
28 Jones, C. *et al.* (1995) *Nature* 376, 145–149
29 Michaelis, R.C. *et al.* (1998) *Am. J. Med. Genet.* 76, 222–228
30 Freudenreich, C.H., Kantrow, S.M. and Zakian, V.A. (1998) *Science* 297, 853–856
31 Richards, R.I. and Sutherland, G.R. (1994) *Nat. Genet.* 6, 114–116
32 Gordenin, D.A., Kunkel, T.A. and Resnick, M.A. (1997) *Nat. Genet.* 16, 116–118
33 Yunis, J.J. and Soreng, A.L. (1984) *Science* 226, 1199–1204
34 Le Beau, M.M. *et al.* (1998) *Genes Chromosomes Cancer* 21, 281–289
35 Siprashvili, Z. *et al.* (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 13771–13776
36 Coquelle, A. *et al.* (1997) *Cell* 89, 215–225

G.R. Sutherland*, E. Baker* *and* ***R.I. Richards§*** *are at the Centre for Medical Genetics, Department of Cytogenetics and Molecular Genetics, Women's and Children's Hospital, Adelaide 5006, and Department of Paediatrics* and Genetics§, University of Adelaide, Adelaide 5000, Australia.*

The genome sequence of *Caenorhabditis elegans* is due to be completed around the end of this year. *C. elegans* will therefore be the first animal to have its genome completely sequenced. To mark this outstanding achievement, we are publishing a series of articles celebrating worm genetics. Articles review the contributions that worm genetics has made to fundamental aspects of biology such as cell death, signal transduction, sex determination and neurobiology.

C. elegans neuroscience: genetics to genome

MARTIN CHALFIE (mc21@columbia.edu)

ERIK M. JORGENSEN (jorgensen@bioscience.utah.edu)

From their earliest experiments, researchers using Caenorhabditis elegans have been interested in the role of genes in the development and function of the nervous system. As the C. elegans Genome Project completes the genomic sequence, we review the accomplishments of these researchers and the impact that the Genome Project has had on their research. We also speculate on future directions in this research that are enabled by the efforts of the Genome Project.

In June 1963, Sydney Brenner wrote to Max Perutz outlining his desire to expand the research activities in the MRC Laboratory of Molecular Biology at Cambridge: 'I have long felt that the future of molecular biology lies in the extension of research to other fields of biology, notably development and the nervous system'[1]. This interest led to his development of the nematode *Caenorhabditis elegans* as a genetic system[2]. Beginning with this initial genetics paper, a major emphasis of researchers using *C. elegans* has been to understand how genes dictate the development and function of the nervous system. Here, we review some of the highlights of research on the *C. elegans* nervous system and describe how the *C. elegans* Genome Project has influenced that research. We emphasize the analysis of the uncoordinated mutants identified by Brenner in his original paper to demonstrate what has been learned. Readers interested in more extensive descriptions of the *C. elegans* nervous system should see Bargmann[3] and relevant chapters in Riddle *et al.*[4]. We have somewhat arbitrarily divided developments in the field into three phases: a genetics era (until the mid 1980s) when most of the major genetic approaches began, the molecular biology era (until the early 1990s) when gene cloning began, and the sequence era (to the present) when the genomic sequence accelerated the molecular analysis of

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nervous system genes. We conclude by describing important areas of study that we believe will emerge in *C. elegans* research in the future.

Phase 1: genetics, development and anatomy (1974–1986)

The attractions of studying *C. elegans* for Brenner were primarily its advantages as a genetic system, specifically, that it is a small organism with a rapid generation time that allows both clonal propagation and genetic crosses. In addition, because the *C. elegans* nervous system has only a small number of neurons (302) with a relatively simple anatomy, cells can be studied individually as well as in the context of an entire nervous system. Moreover, the *C. elegans* nervous system shares many similarities with that of vertebrates. For example, the neurotransmitters in *C. elegans* are largely the same as those in other organisms. Acetylcholine is the major excitatory neurotransmitter at the neuromuscular junction, GABA is an inhibitory neurotransmitter, glutamate functions as a neurotransmitter in the central nervous system, and the monoamines act as modulatory neurotransmitters. The major achievements in the study of *C. elegans* neurobiology that reached fruition in the 1970s and early 1980s were the development of the genetics and the descriptions of the complete cell lineage and cellular anatomy.

Brenner laid the foundation for the genetic analysis of *C. elegans* in his 1974 paper[2]. Most importantly for this review, more than two-thirds of the genes he described in this paper were identified because mutations resulted in uncoordinated movement and so were potentially needed for the development or function of the nervous system. Within a decade of Brenner's initial paper, several extensive screens were conducted to identify mutants with defects in the nervous system and its effectors. Among the first of these were screens for mutants with defective muscles[5,6]. Additional screens were conducted to identify mutants with defects in sensory modalities, for example, in thermotaxis[7], chemotaxis[8], osmotic avoidance[9] and touch[10]. Other screens identified mutations in specific neurotransmitter pathways, specifically, those of dopamine[11], acetylcholine[12–14] and serotonin[15]. Later screens revealed mutants defective in the effector systems for the specific behaviors of egg-laying[15], feeding[16] and defecation[17] and in olfaction[18].

The small size of the animal enabled two monumental efforts in this first period of *C. elegans* research: the description of the cell lineage and the determination of the connectivity of the nervous system. John Sulston and colleagues described all the cell divisions that occur during development from the fertilized zygote to the mature adult hermaphrodite of 959 somatic nuclei[19,20]. John White, Sydney Brenner and colleagues described the anatomy and connectivity of all 302 neurons in the adult hermaphrodite and determined the positions of roughly 5000 synapses, 2000 neuromuscular junctions and 700 gap junctions[21]. These descriptions were possible because both the lineage and neural anatomy are essentially invariant. The invariance of the lineage provided the background with which to analyse mutants with developmental defects, and the invariance of the outgrowth, connectivity and placement of neurons allowed cell function and circuitry to be studied by cellular ablation using a laser microbeam. An early example of the use of laser ablation to study neural circuitry was the analysis of the sensory neurons, interneurons and motor neurons of the touch reflex circuit[22].

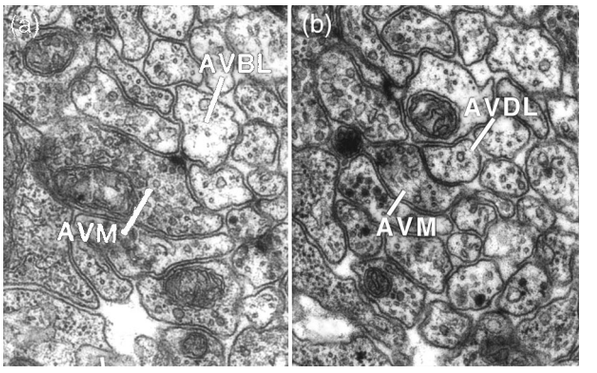

FIGURE 1. Electron micrographs of neuronal connections. (a) A chemical synapses from AVM onto AVBL. (b) A gap junction connecting AVM and AVDL. (Reproduced with permission from Ref. 22).

The anatomical studies revealed that the *C. elegans* nervous system differs in some ways from the nervous systems of vertebrates. As in other nematodes, muscles send processes to the motor neurons, which remain unbranched in the ventral nerve cord. Unlike the synapses of vertebrates or even of *Drosophila*, the synapses in *C. elegans* are simple and lack elaborate postsynaptic specializations (Fig. 1). Even the interneurons are quite simple in structure and lack specialized dendritic arbors or axons. Instead, each neuron usually has a single process that forms en passant synapses with the other processes with which it fasciculates.

Phase 2: molecular biology (1985–1993)

Three research activities greatly accelerated gene cloning in *C. elegans*: (1) the development of a mutator strain by John Collins and Phil Anderson that produced transposon insertions[23], (2) the production of the cosmid physical map by the *C. elegans* Genome Project initiated by John Sulston and Alan Coulson[24], and (3) the development of an effective transformation system by Andy Fire[25]. The mutator strain permitted the tagging of DNA

TABLE 1. Molecular analysis of *unc* genes identified by Brenner (1974)

Product	*unc* gene number
Transcription factor	3, 4, 30, 37, 42, 55
Guidance and outgrowth	5, 6, 33, 40, 44, 53, 69
Channels and possible modifiers	1, 2, 8, 24, 29, 36, 38, 49, 50
Innexin-gap junction	7, 9
Neurotransmitter synthesis and storage	17, 25, 47
Secretion	10, 11, 13, 18, 31, 41, 64
Signal transduction	14, 26, 43, 51
Muscle	15, 22, 45, 52, 54, 60, 68
Not cloned	16, 20, 23, 27, 32, 34, 35, 39, 46, 48, 57, 58, 59, 61, 62, 63, 65, 67, 70, 71, 77

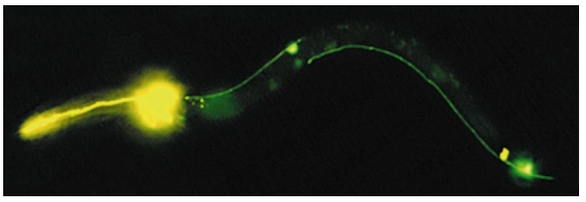

FIGURE 2. Expression of *mec-3::gfp* (green). The animal is also stained with DiI (yellow) which is taken up by some sensory neurons. (Photograph courtesy of Lucinda Carnell and Miriam Goodman.)

so that genes could be identified molecularly. The Genome Project cosmids, coupled with John and Alan's generosity, permitted rapid access to adjacent DNA. The molecular identification could then be confirmed by complementation tests using transformed DNA. The exchange of genomic clones and data about gene identifications enabled the rapid correlation of the genetic and physical maps. As the mapping of the genome became more complete, the transposon tagging of genes became less important and the cosmids from the relevant interval could be directly injected for transformation rescue.

These efforts allowed the cloning of many genes that could be mutated to give an identifiable phenotype. For example, 45 of the 66 *unc* genes, that is, genes that could mutate to an uncoordinated phenotype, described by Brenner[2] have been cloned (additional information on these and other *C. elegans* genes can be obtained by searching the bibliography maintained at Leon Avery's *C. elegans* Web site[26]). These 45 genes provide insights into many of the fundamental questions of neurobiology: six genes encode transcription factors needed for neuronal differentiation, seven encode proteins needed for axon outgrowth and guidance, nine encode or regulate channel or receptor subunits required for cell excitability, two encode gap-junction proteins, three encode proteins needed for neurotransmitter synthesis and storage, seven encode proteins needed for secretion and reuptake of synaptic vesicles, four encode proteins needed for signal transduction, and seven encode proteins needed for muscle contraction (Table 1; these categories are not mutually exclusive, for example, the kinase gene *unc-51* is required for axonal outgrowth).

Although the study of all of these genes has aided our understanding of nervous system function, some have had a critical impact in the field of neurobiology. The first of these, *unc-54*, was cloned well before the Genome Project began; in fact it was the first mutated gene cloned in *C. elegans*. *unc-54* mutants are virtually paralysed and their body wall muscle cells lack most of their thick filaments. By taking advantage of a deletion mutation that produced a shortened mRNA, MacLeod *et al.*[27] cloned and characterized the wild-type allele of what was the first myosin heavy chain gene identified from any organism. The cloning of this gene was particularly important since, given the size of the myosin heavy chain, cell biologists before this time did not have the complete amino acid sequence for the protein. Thus, the cloning of *unc-54* and its subsequent sequencing provided the first structural model for this crucial motor protein[28].

Equally important has been the identification of proteins required for the directed outgrowth of neuronal processes. Foremost of these have been the *unc-6* netrin and its protein partners, the products of the *unc-5* and *unc-40* genes[29–31]. The importance of the *unc-6* work derives not only from the identification of the first of what is now a family of guidance proteins for both vertebrate and invertebrate neurons, but also in the use of the mutant phenotype to demonstrate the *in vivo* importance of the protein in guidance.

One somewhat surprising result from the molecular characterization of the *unc* genes is that several encode transcription factors rather than structural components of the nervous system. Two of the most striking examples are the homeobox transcription factor genes *unc-30* and *unc-4*. Mutations in the *unc-30* gene produce animals that have a shrinker phenotype; such animals simultaneously contract all of their body wall muscles, which causes them to shrink. This phenotype is characteristic of animals with defects in the GABAergic motor neurons that inhibit contraction of the body muscles. In *unc-30* mutants these GABAergic motor neurons fail to differentiate appropriately: they do not make GABA, grow appropriate processes, or form appropriate synapses[32]. Interestingly, despite its requirement for GABA-specific function in ventral cord motor neurons, *unc-30* is expressed in and required for a few non-GABAergic neurons and is not expressed in a few other GABAergic cells. *mec-3*, another homeodomain protein, is required for the differentiation of neurons which sense gentle touch. Like *unc-30*, *mec-3* is expressed in these cells but also in other neurons[33,34] (Fig. 2). Thus, these genes control the differentiation of groups of related neurons, but their expression patterns suggest that combinatorial regulation of neuronal differentiation is important.

The *unc-4* gene encodes a homeodomain protein related to UNC-30 (both are of the orthodenticle class) and regulates an even narrower set of features for a class of neurons – the choice of synaptic partners[35]. *unc-4* mutants cannot go backward, a defect attributable to the loss or inappropriate function of the A-type motor neurons. Electron microscopic reconstruction[36] of the neurons in these mutants revealed that the A motor neurons appeared to grow out correctly and make appropriate neuromuscular connections. A striking defect is that some of these cells fail to receive their normal interneuron connections. Instead they receive the same set of synapses as the B motor neurons (a set of motor neurons that are needed for forward movement). The problem is not that the cells are incapable of receiving synaptic inputs or that the cells are incorrectly positioned so they cannot find their correct partners. Rather, the defect appears to be a failure to form synapses with the appropriate adjacent cells.

In his 1974 paper Brenner also found several genes that could be mutated to give resistance to drugs thought to interfere with the action of the neurotransmitter acetylcholine. Tetramisole acts on nematode acetylcholine receptors and three of the four genes that Brenner identified by drug resistance encode subunits of the acetylcholine receptors of the muscle[37]. Brenner also found that *unc-17* mutations conveyed resistance to aldicarb, a drug that blocks the action of acetylcholinesterase and causes a buildup of acetylcholine in the synaptic cleft.

The molecular characterization of the *unc-17* gene identified the first vesicular acetylcholine transporter and revealed that it was related to the monoamine transporter[38]. Interestingly, *unc-17* shares a common initial exon with *cha-1*, the gene for the acetylcholine synthetic enzyme choline acetyltransferase. This complex gene structure is conserved from nematodes to humans[39–41].

The *unc-17* mutations cause aldicarb resistance presumably by reducing the amount of acetylcholine at cholinergic synapses. Subsequent researchers have found that almost 40 genes can also mutate to produce aldicarb resistance[42]. Most of these genes are needed for synaptic vesicle release and recycling. For example, the SNARE complex is a group of proteins thought to mediate fusion of synaptic vesicles with the plasma membrane[43]. Mutations in the genes encoding these proteins have been identified in *C. elegans*, including the genes for syntaxin[44,45], synaptobrevin[46], UNC-18 (Ref. 47) and synaptotagmin[48,49]. Perhaps the most intriguing gene whose product appears to be needed for synaptic vesicle release is *unc-13*. *unc-13* encodes a large protein that contains diacylglycerol and Ca^{2+}-binding sites[50]. Mammalian homologues, called *MUNC13* genes, have also been cloned[51], and the MUNC13 proteins bind to syntaxin[43,52]. Although the exact role of UNC-13 in synaptic vesicle release is as yet unknown, its subcellular localization and the presence of calcium binding motifs suggest that it may be an integral component of the release machinery.

This review has emphasized the impact of the mutants first found by Brenner, but several other studies have contributed broadly to the neurosciences. Foremost have been the studies on the molecular basis of programmed cell death[53]. In the area of cell differentiation, *C. elegans* research led to the identification of two new types of homeodomain transcription factors that control neuronal cell fates, the POU type defined by *unc-86* (Ref. 54) and the LIM type defined by *mec-3* (Refs 55, 56). In sensory neurobiology, the study of genes needed for touch sensitivity led to the first molecular model of eukaryotic mechanosensation[57] and the cloning of the *odr-10* gene led to the identification of the first receptor for an identified odorant[58]. Finally, work in an area outside *C. elegans* neurobiology is having an impact on the study of human neurobiology and disease. Specifically, the finding that a suppressor of the *C. elegans lin-12* gene, *sel-12*, encodes a homologue of presenilin 1, a gene implicated in Alzheimer disease, has raised the possibility that improper function of the *lin-12/Notch* signaling pathway may underlie some of the problems in this disease[59].

Phase 3: sequence (1994–present)

In 1990 a collaborative effort between the laboratories of Bob Waterston and John Sulston to sequence the entire *C. elegans* genome was begun. By about 1994 a significant fraction of genomic sequence was available to the community and these data began to change the way *C. elegans* science was performed. Now with 80% of the genomic sequence complete (and the remainder in various stages of completeness), we can begin to examine the sequence information to gain a fuller description of the *C. elegans* nervous system and use the information to more readily study gene function in the nervous system.

One question we can begin to address is how similar or different the *C. elegans* nervous system is to other nervous systems at a molecular level. While many genes affecting mammalian nervous systems are found in *C. elegans*, for example, the animal has homologues for agrin, MuSK and the *LIS-1* lissencephaly gene (C. Ma, G. Caldwell and M. Chalfie, unpublished), some differences are already apparent. First, *C. elegans* does not appear to have genes encoding voltage-gated sodium channels. Because electrophysiological studies of the larger nematode *Ascaris suum* suggested that nematode neurons might not have Na^+-based action potentials[60], this finding was not completely unexpected. Nonetheless, the genome data appear to confirm this hypothesis. The absence of a voltage-sensitive Na^+ channel, however, does not mean that *C. elegans* is incapable of producing action potentials. As the genome sequence predicts several voltage-sensitive Ca^{2+} channel genes, *C. elegans* may use a combination of Ca^{2+}-based action potentials and graded potentials for neuronal signaling. By contrast, previous studies had suggested that nematodes would not have NMDA-type glutamate receptors, but the genome sequence clearly indicates that *C. elegans* has at least two (Villu Maricq, pers. commun.).

Second, *C. elegans* lacks connexins, the proteins that form gap junctions in vertebrates. Virtually all of the *C. elegans* nervous system is coupled through gap junctions, so other proteins must be forming these communication channels. These functions are assumed by the innexin family proteins in *C. elegans*[61]. Two of these proteins are encoded by the *C. elegans unc-7* and *unc-9* genes[62,63], which were identified by Brenner as mutants with severe locomotory defects; a third, *eat-5*, disrupts function of the pharynx[16]. Innexin proteins can form dye-permeant junctions when expressed in cultured cells, thereby proving that they are gap junction proteins[64]. The replacement of connexins by innexins probably reflects an essential difference between vertebrates and invertebrates, because these same proteins are found in insects as well as nematodes.

Beyond the comparative studies, a second issue that can be addressed using the complete genome sequence is the molecular complexity of the nervous system. Despite the small number and simple form of its neurons, the *C. elegans* nervous system is surprisingly complex at a molecular level. Even though the nervous system has only 302 neurons in 118 classes[21], at least 66 genes encode subunits of the ligand-gated superfamily of ion channels, ten genes encode glutamate receptor subunits (Villu Maricq, pers. commun.), at least 80 genes encode potassium channels (Lawrence Salkoff, pers. commun.), and five genes encode alpha subunits of voltage-sensitive Ca^{2+} channels.

Finally, the genome sequence has changed and accelerated the way we characterize genes identified using forward genetics. Frequently, a gene can be cloned by simply making the best guess among candidate genes in a given interval and using that candidate for transformation experiments. Once a gene has been identified, its sequence can be used to obtain other homologues in *C. elegans*. This search for homologues has become a routine aspect of gene analysis in *C. elegans*. For example, the genes *deg-1* and *mec-4* can be mutated to give a similar neurodegeneration phenotype. When they were sequenced, they were found to encode novel, yet

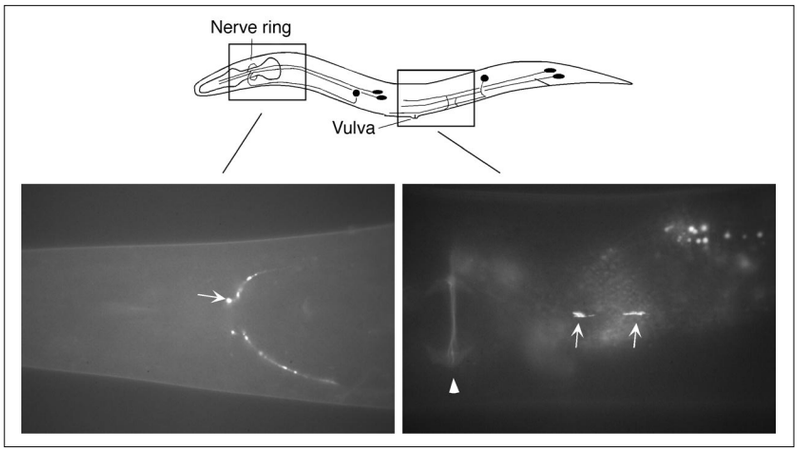

FIGURE 3. Chemical synapses made by touch receptor neurons (the green fluorescent cells in Fig. 2) as identified by the localization of fluorescence from a *snb-1::gfp* fusion. The *snb-1* gene encodes synaptobrevin (also called VAMP). (Courtesy of Anneliese Schaefer and Mike Nonet.)

similar, proteins that were named degenerins[65,66]. The degenerins were later shown to be related to a family of channel proteins that include the vertebrate amiloride-sensitive Na^+ channel subunits and the snail FMRFamide-gated ion channel. Because null mutations in *mec-4* cause worms to be touch insensitive, MEC-4 is likely to be part of a mechanosensory channel. The *C. elegans* genome sequence predicts at least 23 genes encoding similar proteins. Since some of these are similar to MEC-4 and others are more divergent, these proteins may provide a variety of ion transport functions in addition to mechanosensation in different cells. Another example is the *unc-47* gene, whose product was the first identified vesicular GABA transporter[67]. Even though UNC-47 is unlike previous neurotransmitter transporters, homology searches of the genomic sequence identified 12 predicted proteins similar to it. The study of these homologues should provide insights into the general function of this new class of transport proteins.

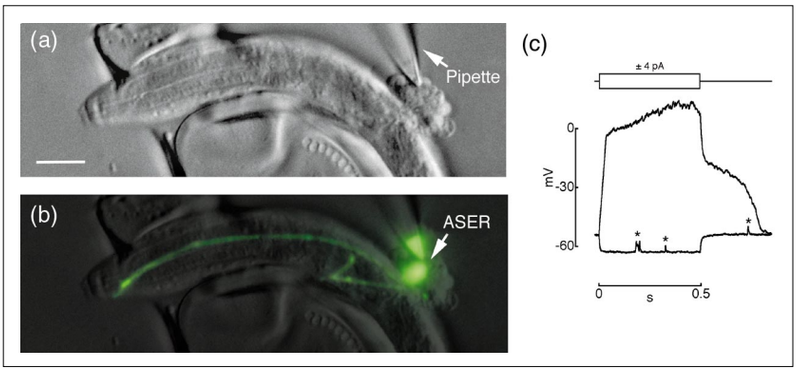

FIGURE 4. Electrical recordings from the ASER neuron. (a) Differential interference contrast micrograph showing the recording pipette sealed onto the ASER cell body. (b) Fluorescence micrograph of the same field as in (a) showing the expression of GFP in the ASER cell body. Note that some of the cell contents have entered the recording pipette. (c) Membrane voltage during current injection. A current pulse of 4 pA depolarizes the cell by 60 mV, whereas a pulse of –4 pA hyperpolarizes the cells by less than 10 mV. (Adapted with permission from Ref. 82)

Such sequence gazing is usually confined to searches for homologues of known proteins, but careful searches can also reveal new molecules that function in the nervous system. For example, an analysis of seven-pass transmembrane receptors in the worm revealed 40 members in several novel classes[68]. Because some of these molecules were expressed in chemosensory neurons, they are strong candidates for olfactory or gustatory receptors. However, some of these receptors are not expressed in sensory neurons. Those expressed in other cells are presumed to be acting as receptors for novel neurotransmitters or hormones and thus may identify new signaling mechanisms in the nervous system.

Several technical developments have strengthened the importance of the sequence data. One particularly useful example, and one that has become an integral part of the Genome Project, is the systematic isolation and characterization of cDNAs (Refs 69, 70). A second example, the development of the *Aquorea victoria* green fluorescent protein (GFP) for cellular and subcellular localization[71], has provided a powerful means of analysing gene expression (Fig. 3). The Troeml *et al.*[68] experiments described in the previous paragraph were made more meaningful by the thorough examination of expression patterns using GFP fusions. A third example, is the development of reverse genetic techniques in *C. elegans*. Specifically, deletions of a particular gene can be generated by screening for imprecise excision of transposons[72] or by screening for deletions generated by chemical mutagens[73]. For example, an analysis trimeric G-protein function in the worm has been conducted by systematically mutating the cognate genes encoding these signaling molecules[73–75]. Most recently, the discovery that injection of double-stranded RNA (interference RNA or RNA_i) could prevent normal gene activity[76] promises to be a rapid means of estimating the null phenotype for any gene.

Phase 4: the future

The use of the complete sequence of the *C. elegans* genome to inform and drive forward and reverse genetic strategies will occupy worm neurobiologists for some time. In addition, as the genomes of other organisms are sequenced, questions concerning the evolution of the nervous system can be addressed. But what new directions are emerging that will define the future of *C. elegans* neurobiology? The genome sequence can be put to two immediate uses. The first is the development of methods to characterize global gene expression and

its alteration by mutation. One current effort by Yuji Kohara (pers. commun.) aims to examine the *in situ* hybridization pattern of all identified cDNAs. In another effort, Chuck Ma and Martin Chalfie (unpublished) are developing subtraction library methods to identify genes that are differentially expressed in the wild type and a mutant. In addition, Stuart Kim (pers. commun.) is examining the use of chips with DNAs representing all of the predicted genes in *C. elegans* that can be hybridized with wild-type and mutant mRNAs.

The second use of the sequence information is in the development of methods to identify interacting sets of proteins. Already under discussion are attempts to use yeast two-hybrid technology to examine protein interactions among all of the predicted proteins in the genome (Marc Vidal, pers. commun.). Once interacting partners are isolated, only a small amount of sequence will be needed to identify the interacting gene. Similarly, we envision that protein biochemistry will be increasingly important for *C. elegans* research. As protein complexes are isolated from the animal, mass spectroscopy can be used to identify the precise proteins in the complex simply by comparing the molecular weight with that predicted from the sequence data.

Ultimately, however, understanding a nervous system requires understanding its function. Even if we had the expression patterns of all the genes in the animal, we would be far from understanding how this small (although obviously not simple) nervous system works. The challenge for neurobiologists (and geneticists) is to understand more of what all 302 neurons do. Four major questions need to be addressed: how do synapses develop, what is the neurotransmitter and receptor at every synapse, what are the electrophysiological characteristics of these synapses, and how do small circuits function to regulate the behavior of an intact animal?

First, we need to understand how the nervous system develops. GFP markers that label specific growth cones can provide us with the specific timing and sequence of developmental events in the formation of the nervous anatomy. Eventually we would like to know with which axons each neuron fasciculates as it develops, and at which landmarks growth cones change their behavior. In addition, we need to establish whether there are changes in synaptic partners as the nervous system develops. The complete reconstruction gave us information about the connectivity of the adult nervous system; however, we know that rewiring occurs during development[77]. To some extent such information can be gathered by observing synaptic varicosities during development that can be visualized by tagging synaptic vesicles with GFP (Mike Nonet, pers. commun.; Fig. 3). However, to determine the precise connectivity during development will probably require the analysis of electron micrographs.

The second research area will be the characterization of neurotransmitter and neuromodulator utilization within the nervous system. In general, few of the chemical signals acting on nerve cells are known in the animal. However, several enzymes required for neurotransmitter synthesis, metabolism and storage have been identified in the genome sequence and their localization will help in the characterization of the nervous system. In addition, the genome sequence predicts several families of potential neuropeptides (Anne Hart, pers. commun.), but only one gene, encoding several FMRFamide-like peptides, has been studied[78,79]. The postsynaptic receptors to these neurotransmitters can be identified by tagging these proteins with GFP. Together, these experiments will predict, but not establish, whether a synapse is excitatory or inhibitory.

A complete understanding of the molecules that mediate transmission at a particular synapse will not determine how such a synapse will function in an intact circuit. Thus, the third area of study must be the analysis of the electrophysiology of *C. elegans* neurons to develop electrophysiological tools for *C. elegans*. The small size of *C. elegans* neurons has prevented such an analysis in the past, but recent developments suggest that electrophysiological studies may be more routine in the future. Pioneering efforts by Leon Avery and colleagues demonstrated that field recordings could be made from pharyngeal muscle that resolved both muscle-derived and synaptic currents[80]. More recent studies have demonstrated that patch clamping pharyngeal muscle[81], body muscle (J. Richmond and E. Jorgensen, unpublished), and even neurons[82] is possible (Fig. 4). In the next few years we expect that these and other techniques such as voltage-sensitive or calcium-sensitive fluorescence will be perfected and the electrophysiological characterization of the *C. elegans* nervous system will be well under way.

Finally, experiments to identify the function of defined circuits in the regulation of behavior are needed. Traditionally, these experiments have relied on killing cells by laser microsurgery. Newer methods, however, may provide better ways of perturbing cell activity. For example, the expression of constitutively-active potassium channels or ligand-gated chloride channels could depress the activity of a particular cell (David Weinshenker and Jim Thomas, pers. commun.; Joe Dent, pers. commun.) and the expression of activated cation channels can increase the activity of a cell in a circuit (Villu Maricq, pers. commun.). These experiments could provide a functional map of the nervous system that can be related to the neural circuitry. Eventually, we believe that a synthesis joining the anatomical, developmental and electrophysiological data will provide a general model of how a nervous system functions.

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published, is a fitting example of this cooperative spirit. Virtually every research project in the field has benefited, directly or indirectly, from the Genome Project. As the beneficiaries of this work, we are grateful not only for the vast amount of information and materials that the Genome Project has provided us, but also for the spirit in which the work has and is being conducted. They bring credit to our enterprise.

References

1 Brenner, S. (1988) in *The Nematode Caenorhabditis elegans* (Wood, W.B., ed.), pp. 9–13, Cold Spring Harbor Laboratories
2 Brenner, S. (1974) *Genetics* 77, 71–94
3 Bargmann, C.I. (1993) *Annu. Rev. Neurosci.* 16, 47–71
4 Riddle, D.L., Blumenthal, T., Meyer, B.J. and Priess, J.R. (1997) *C. elegans II*, Cold Spring Harbor Laboratories
5 Waterston, R.H., Thomson, J.N. and Brenner, S. (1980) *Dev. Biol.* 77, 271–302
6 Zengel, J.M. and Epstein, H.F. (1980) *Cell Motil.* 1, 73–97
7 Hedgecock, E.M., Culotti, J.G. and Hall, D.H. (1990) *Neuron* 4, 61–85
8 Lewis, J.A. and Hodgkin, J.A. (1977) *J. Comp. Neurol.* 172, 489–510
9 Culotti, J.G. and Russell, R.L. (1978) *Genetics* 90, 243–256
10 Chalfie, M. and Sulston, J. (1981) *Dev. Biol.* 82, 358–370
11 Sulston, J., Dew, M. and Brenner, S. (1975) *J. Comp. Neurol.* 163, 215–226
12 Culotti, J.G., Von Ehrenstein, G., Culotti, M.R. and Russell, R.L. (1981) *Genetics* 97, 281–305
13 Johnson, C.D. *et al.* (1981) *Genetics* 97, 261–279
14 Rand, J.B. and Russell, R.L. (1984) *Genetics* 106, 227–248
15 Trent, C., Tsung, N. and Horvitz, H.R. (1983) *Genetics* 104, 619–647
16 Avery, L. (1993) *Genetics* 133, 897–917
17 Thomas, J.H. (1990) *Genetics* 124, 855–872
18 Bargmann, C.I., Hartwieg, E. and Horvitz, H.R. (1993) *Cell* 74, 515–27
19 Sulston, J.E. and Horvitz, H.R. (1977) *Dev. Biol.* 56, 110–156
20 Sulston, J.E., Schierenberg, E., White, J.G. and Thomson, J.N. (1983) *Dev. Biol.* 100, 64–119
21 White, J.G., Southgate, E., Thomson, J.N. and Brenner, S. (1986) *Philos. Trans. R. Soc. London. (B. Biol. Sci.)* 314, 1–340
22 Chalfie, M. *et al.* (1985) *J. Neurosci.* 5, 956–964
23 Collins, J., Saari, B. and Anderson, P. (1987) *Nature* 328, 726–728
24 Coulson, A., Sulston, J., Brenner, S. and Karn, J. (1986) *Proc. Natl. Acad. Sci. U. S. A.* 83, 7821–7825
25 Fire, A. (1986) *EMBO J.* 5, 2673–2680
26 Leon Avery's *C. elegans* Web site at http://www.eatworms.swmed.edu
27 MacLeod, A.R., Karn, J. and Brenner, S. (1981) *Nature* 291, 386–390
28 McLachlan, A.D. and Karn, J. (1983) *J. Mol. Biol.* 164, xx–xx
29 Ishii, N. *et al.* (1992) *Neuron* 9, 873–881
30 Leung-Hagesteijn, C. *et al.* (1992) *Cell* 71, 289–299
31 Chan, S.S. *et al.* (1996) *Cell* 87, 187–195
32 Jin, Y., Hoskins, R. and Horvitz, H.R. (1994) *Nature* 372, 780–783
33 Way, J.C. and Chalfie, M. (1989) *Genes Dev.* 3, 1823–1833
34 Mitani, S. *et al.* (1993) *Development* 119, 773–783
35 Miller, D.M. *et al.* (1992) *Nature* 355, 841–845
36 White, J.G., Southgate, E. and Thomson, J.N. (1992) *Nature* 355, 838–841
37 Fleming, J.T. *et al.* (1997) *J. Neurosci.* 17, 5843–5857
38 Alfonso, A. *et al.* (1993) *Science* 261, 617–961
39 Alfonso, A. *et al.* (1994) *J. Mol. Biol.* 241, 627–630
40 Bejanin, S., Cervini, R., Mallet, J. and Berrard, S. (1994) *J. Biol. Chem.* 269, 21944–21947
41 Erickson, J.D. *et al.* (1994) *J. Biol. Chem.* 269, 21929–21932
42 Miller, K.G. *et al.* (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 12593–12598
43 Sollner, T. *et al.* (1993) *Nature* 362, 318–324
44 Ogawa, H. *et al.* (1998) *J. Biol. Chem.* 273, 2192–2198
45 Saifee, O., Wei, L. and Nonet, M.L. (1998) *Mol. Biol. Cell.* 9, 1235–1252
46 Nonet, M.L. *et al.* (1998) *J. Neurosci.* 18, 70–80
47 Hosono, R. *et al.* (1992) *J. Neurochem.* 58, 1517–1525
48 Nonet, M.L., Grundahl, K., Meyer, B.J. and Rand, J.B. (1993) *Cell* 73, 1291–1305
49 Jorgensen, E.M. *et al.* (1995) *Nature* 378, 196–199
50 Maruyama, I.N. and Brenner, S. (1991) *Proc. Natl. Acad. Sci. U. S. A.* 88, 5729–5733
51 Brose, N., Hofmann, K., Hata, Y. and Sudhof, T.C. (1995) *J. Biol. Chem.* 270, 25273–25280
52 Betz, A., Okamoto, M., Benseler, F. and Brose, N. (1997) *J. Biol. Chem.* 272, 2520–2526
53 Metzstein, M., Stanfield, G. and Horvitz, H.R. (1998) *Trends Genet.* 14, 410–416
54 Finney, M., Ruvkun, G. and Horvitz, H.R. (1988) *Cell* 55, 757–769
55 Way, J.C. and Chalfie, M. (1988) *Cell* 54, 5–16
56 Freyd, G., Kim, S.K. and Horvitz, H.R. (1990) *Nature* 344, 876–879
57 Gu, G., Caldwell, G.A. and Chalfie, M. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 6577–6582
58 Sengupta, P., Chou, J.H. and Bargmann, C.I. (1996) *Cell* 84, 899–909
59 Levitan, D. and Greenwald, I. (1995) *Nature* 377, 351–354
60 Davis, R.E. and Stretton, A.O. (1989) *J. Neurosci.* 9, 415–425
61 Phelan, P. *et al.* (1998) *Trends Genet.* 14, 348–349
62 Starich, T.A. *et al.* (1996) *J. Cell Biol.* 134, 537–548
63 Barnes, T.M. and Hekimi, S. (1997) *J. Neurochem.* 69, 2251–2260
64 Phelan, P. *et al.* (1998) *Nature* 391, 181–184
65 Chalfie, M. and Wolinsky, E. (1990) *Nature* 345, 410–416
66 Driscoll, M. and Chalfie, M. (1991) *Nature* 349, 588–593
67 McIntire, S.L. *et al.* (1997) *Nature* 389, 870–876
68 Troemel, E.R. *et al.* (1995) *Cell* 83, 207–218
69 Waterston, R. *et al.* (1992) *Nat. Genet.* 1, 114–123
70 Kohara, Y. (1996) *Tanpakushitsu Kakusan Koso* 41, 715–720
71 Chalfie, M. *et al.* (1994) *Science* 263, 802–805
72 Zwaal, R.R. *et al.* (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 7431–745
73 Jansen, G., Hazendonk, E., Thijssen, K.L. and Plasterk, R.H. (1997) *Nat. Genet.* 17, 119–121
74 Zwaal, R.R. *et al.* (1996) *Cell* 86, 619–629
75 Zwaal, R.R., Mendel, J.E., Sternberg, P.W. and Plasterk, R.H. (1997) *Genetics* 145, 715–727
76 Fire, A. *et al.* (1998) *Nature* 391, 806–611
77 White, J.G., Albertson, D.G. and Anness, M.A. (1978) *Nature* 271, 764–766
78 Rosoff, M.L., Burglin, T.R. and Li, C. (1992) *J. Neurosci.* 12, 2356–2361
79 Rosoff, M.L., Doble, K.E., Price, D.A. and Li, C. (1993) *Peptides* 14, 331–338
80 Raizen, D.M. and Avery, L. (1994) *Neuron* 12, 483–495
81 Davis, M.W. *et al.* (1995) *J. Neurosci.* 15, 8408–8418
82 Goodman, M.B., Hall, D.H., Avery, L. and Lockery, S.R. (1998) *Neuron* 20, 763–772

M. Chalfie *is in the Department of Biological Sciences, 1012 Fairchild, Mail Code 2446, Columbia University, 1212 Amsterdam Avenue, New York, NY 10027, USA.*
E.M. Jorgensen *is in the Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112-0840, USA.*