The basic process of chemotaxis in Escherichia coli and other flagellated bacteria is very well understood. As reviewed by Howard Berg of Harvard University and Sandy Parkinson of University of Utah, it involves a biased random walk or run-and-tumble pattern of movement. The bacterium swim in a straight line (a run), driven by the counter-clockwise rotation of flagella. At times, however, one or more of the flagellar motors will change direction, leading to a tumbling event. The organism then proceeds on another run but in a new, randomly determined direction. To control the frequency of tumble events, the bacterium samples the chemical composition of its medium continuously and compares to past compositions stored as a chemical memory in receptor adaptation sites. A decline in the concentration of chemical attractants will favor a tumble event, thereby shortening runs down the attractant gradient and creating an overall bias of movement up the gradient. The chemosensory pathway that detects attractants is quite sensitive, with the ability to respond to a 0.1% change in concentration. At the same time it can operate over a large concentration range spanning five orders of magnitude.

Keiichi Namba of Osaka University presented an overview of a number of remarkable structural studies focusing on the flagella and associated proteins. The structure of the flagellum itself, consisting of an assembly of flagellin monomers into a filament, was solved using x-ray crystal structures in combination with cryoelectron microscopy of intact filaments using advanced image-processing techniques. Namba and colleagues have also used this approach to produce a structure for the flexible “hook” region. The hook consists of the flagellar protein FlgE and acts as a universal joint connecting the intracellular motor with the main propeller region of the flagellum.

The protein Flil sits at the base of the flagellum and transports protein monomers into the central channel of the filament during flagellar biogenesis. The structure and subunit organization of this protein is similar to that of the mitochondrial F0-F1 ATPase, suggesting that this transport process uses ATP in a similar manner, perhaps to turn a rotary motor involved in transporting monomers from the cytoplasm into the flagellar channel.

The principle behind the motor that drives flagellar rotation is apparently also that of an ion-driven machine that also makes use of the transmembrane proton gradient to energize flagellar rotation. The speed of rotation, which can reach hundreds of revolutions per second, is proportional to the proton-motive force across the membrane under a variety of conditions, and at least at low speeds operates at a constant torque. However the molecular mechanism of the proton-driven rotation is not known.

The switching of the rotary motor between the clockwise (tumble) and counterclockwise (run) states is controlled by a chemosensory pathway initiated by transmembrane receptors for various attractants and repellents (see Fig. 1). The structure of these receptors is known in some detail. The basic unit is a homodimer with an extracellular ligand-binding site, four transmembrane helices (two from each subunit), and another intracellular bundle of four coiled-coil helices from the two C termini in a helix-loop-helix arrangement (again, two from each subunit). As discussed by Joe Falke (University of Colorado), a key structural change in the receptor upon binding a substrate is a subtle 1.5-Å displacement of one of the transmembrane helices in a piston-like motion that carries the transmembrane signal. Falke presented additional evidence that a different type of motion transmitted by subtle rearrangements of
helix packing within the extended cytoplasmic domain carries receptor signals to the cytoplasmic CheA kinase docked at the distal tip of the four-helix bundle. This model explains receptor signals triggered by attractant binding and by covalent methylation of the receptor adaptation sites, which decreases ligand sensitivity and activates the kinase by stabilizing helix–helix interactions. Parkinson discussed the role of the “HAMP” region of the receptor that links the transmembrane and cytoplasmic helix bundles. He suggested that changes in the internal tension of the HAMP structure could be used to transmit signals between the transmembrane and cytoplasmic domains.

CheA itself is a homodimer in which each subunit possesses five domains, whose structures have been solved individually. The relative positions and orientations of these five domains in the full-length CheA protein were explored in two of the talks. Brian Crane (Cornell University) used pulsed ESR in combination with structures of multicomponent crystals to study the relative positions of the CheA domains and their interactions with the adaptor protein CheW. He found that critical residues identified by mutagenesis map to the presumed interfaces between CheW and the receptor, and to areas where the CheA protein contacts itself. Rick Dahlquist (University of California, Santa Barbara) used NMR to study the interactions among CheA domains and how these change upon phosphorylation. His data are consistent with the existence of important interactions between the P3 (“dimerization”) and P4 (“kinase”) domains. The P1 (phosphorylated) domain interacts with P3P4 at sites distinct from the P4 catalytic site and from the phosphorylated histidine of P1. This histidine is thereby exposed, permitting the transfer of the phosphate to the motor-regulating protein CheY. Joe Falke used a different approach—cysteine modification of the protein followed by functional assays—to map surfaces for the interaction of P1 and P4, the docking of CheA and CheW, and the binding of CheA to the receptor. These domains appear to be highly conserved among CheA homologues.

The receptors are not distributed randomly at the cell surface but are aggregated within one or more clusters, each containing thousands of proteins. As described by Victor Sourjik (University of Heidelberg), these clusters are typically at the poles of the cell but can also be found less frequently at up to eight different lateral sites corresponding to points of cell division. This clustering facilitates interactions between receptors, either of the same type or of different types, and between receptors and their associated proteins. The clusters are not physically associated with the flagellar motors, as diffusion of the CheY protein through the cytoplasm from receptor complexes to the motors is sufficient to carry the tumble signal from receptor complexes to the motors.

The precise arrangement of receptors within the cluster is uncertain. Crystals of the cytoplasmic domains of the receptors from E. coli form “trimers of dimers” and this may comprise the basic unit within the cluster, as suggested by evidence from the Parkinson laboratory. However Crane reported that the receptors from another bacterium, Thermotoga maritima, do not appear to crystallize as trimers but instead form a more extensive “forest” of dimers packed side to side, suggesting that the trimer-of-dimers arrangement may not be universal. Interactions between receptors appear to be sufficient for clustering, although the presence of the CheA and CheW, another receptor-associated protein, can enhance their formation.

Whatever the structure, the clusters appear to be important for cooperative interactions among receptors. This cooperativity can give rise to a significant signal in response to occupancy of a very small number of receptors with ligand. This enhances the sensitivity to chemical signals and enables the cells to respond to molecules for which there are few receptors. As discussed by Dennis Bray (University of Cambridge), these phenomena can be simulated in mathematical models that include up to 65 reactions for aspartate signaling alone. Using experimentally determined reaction rates and assuming cross-talk among the receptors, the models faithfully simulate the basic behavior of the
system in *E. coli*. However, the model parameters need to be tweaked a bit to account for some of the more detailed phenomena.

Ann Stock (Rutgers University) described the similarities between chemotaxis and other signal transduction pathways in bacteria. In many of these, environmental signals are sensed by receptors coupled to kinases that transfer phosphate from a histidine on the kinase itself to an aspartate group on a transcription factor. She discussed the interaction of regulators of the OmpR/PhoB family that form an active state composed of dimers that can bind to DNA and control transcription. The dimers have a symmetrical arrangement of their phosphoryl-receiver domains but a tandem, head-to-tail structure of their DNA-binding domains. She proposed that different proteins of this family have different inactive-state conformations that can respond to different signals, but similar active-state structures.

II. Eukaryotic Chemotaxis

Although the basic goal of chemotaxis in eukaryotic cells is the same as in bacteria, namely movement toward a chemical attractant, the mechanisms underlying the responses are very different. While bacteria can swim in three dimensions toward a nutrient, movement of eukaryotic cells is more akin to crawling along a surface. Rather than using flagella as propellers, these cells use actin-driven extensions of the cytoplasm to reach their destination.

The basic chemosensing strategy is also different. While bacteria convert spatial gradients to temporal concentration changes by moving rapidly through space, eukaryotic cells respond directly to concentration differences over the dimensions of the cell. How this is accomplished is not completely understood. However Tobias Meyer (Stanford University) presented evidence for a model in which the extensions of the cytoplasm occur as stochastic events driven by interaction of chemical signals with receptors located near the head end of the cell. Concentration differences of an attractant from one side of the cell to the other will cause the cell to turn in the direction of the higher concentration, leading to a predominant movement in the direction of the attractant. This produces either a biased random walk for shallow gradients or a smoother, directed movement when the gradients are steep.

Peter Devreotes (Johns Hopkins University) described the events known to occur in the signal transduction process (see Fig. 2). The two best-studied examples are mammalian neutrophils and the amoeba *Dictyostelium discoideum*. The receptors themselves are seven membrane-spanning proteins and are coupled to heterotrimeric G proteins. In neutrophils these receptors include those that respond to bacterial formylated peptides, while the best understood system in *Dictyostelium* is the receptor for cAMP that governs the aggregation response. Receptor activation leads to a number of cytoplasmic events, including the local activation and recruitment of PI3 kinase to the membrane and the generation of PIP3. Dephosphorylation of PIP3 is largely through the membrane-bound PTEN phosphatase that is selectively removed from the leading edge of the cell in response to an attractant but remains concentrated at the rear. This results in an accumulation of PIP3 at the front of the cell (and depletion from the back), leading to the polarized recruitment of a number of signaling proteins to the front edge through interactions of pleckstrin homology (PH) domains with the PIP3. The importance of this system is underscored by the behavior of PTEN-knockout cells, which tend to extend pseudopods at random positions, preventing efficient chemotaxis. However blocking PI3 kinase does not prevent chemotaxis, demonstrating the existence of alternative signaling pathways.

![Signal transduction pathways in eukaryotic chemotaxis.](image)

**Figure 2.** Signal transduction pathways in eukaryotic chemotaxis. The compass component consists of G protein–coupled receptors and other upstream components that can sense spatial gradients of chemoattractant concentration. Activation of receptors leads to the generation of signals through several interacting pathways. PI3K (kinase) and PTEN (phosphatase) control the concentration of PIP3 in the membrane. These lipids help to build a scaffold of signaling molecules through interactions with PH domain–containing proteins such as PKB. Chemoattractants can also influence PKB activity through activation of the TOR2 complex. A third pathway involving phospholipase A2 is also activated by chemoattractant binding. These events control a set of coordinated changes in the actin cytoskeleton leading to polarization of the cell, increased motility, and movement toward higher attractant concentrations. Figure courtesy of Peter Devreotes, Johns Hopkins University.
Small monomeric G proteins, including Rac and Cdc42, form another set of signaling molecules. How the G proteins are activated in response to receptor activation is not well understood. However some of their downstream targets have been identified. These include the proteins Scar and WASP, which in turn can regulate a number of cellular processes that lead to actin polymerization.

As in the case of the bacterial system, the high sensitivity of the response to attractants is thought to involve an amplification process within the signaling pathway. In the eukaryotic system, a positive feedback loop is presumed to underlie this amplification, although the nature of the feedback is unclear. One possibility is that actin polymerization drives not only movement toward the signal but also up-regulates one of the earlier steps in the response, such as the PI3 kinase. Meyer, however, presented data supporting a shorter feedback loop in which the kinase is further activated by its product, PIP3, presumably through the binding of additional proteins to the negatively charged phospholipids.

A number of proteins are involved in the transduction of these early signaling events to the eventual formation and extension of lamellipodia. Miguel Vicente-Manzanares (University of Virginia) emphasized the role of paxillin, a regulatory protein found in the membrane-associated scaffold that connects to actin filaments. Paxillin is regulated by phosphorylation, and it can activate the small G-protein Rac. It appears to be essential for the property of dynamic adhesion that allows the migrating cell to make and then break contacts with its substrate. Laura Machesky (University of Birmingham) discussed the parallel activation of two pathways involving the soluble G proteins Rac and Cdc42. In both cases the effector proteins (Scar in the case of Rac or WASP in the case of Cdc42) can in turn regulate Arp2/3, an actin-organizing protein complex. The Rac→Scar pathway seems to be essential for normal lamellipod formation but not for cell migration per se. Furthermore cells lacking Scar1 can also form normal “dorsal ruffles,” another structure based on actin. Mechevsky also described the role of MIM-B, a protein that is involved in both the binding of Rac and the bundling of actin filaments into lamellipodia. Tatiana Svitkina (University of Pennsylvania) talked about the roles of Arp2/3 and the formin family protein mDia2 in the control of actin polymerization; Arp2/3 promotes the nucleation of the actin filaments and mDia2 prevents the capping of the filaments. These two events are both essential for the normal elongation of actin into filopodia and lamellipodia.

Conspicuously absent from most of the signal transduction schemes discussed at the meeting was a role for Ca2+. However, John Evans (University of Colorado) presented evidence for the importance of cytoplasmic Ca2+ in chemotaxis. Chelation of extracellular Ca2+ collapsed lamellipodia and decreased the accumulation of PIP3 at the leading edge of the cell. Similar results were obtained with LaCl3, which can inhibit Ca2+ entry into the cell. This suggests that Ca2+ influx may be involved in the orchestration of the chemotactic response.

Many recent advancements in this field have been driven by the development of new technologies for investigating protein activities and protein–protein interactions in living cells. These generally involve sophisticated optical techniques. Klaus Hahn (University of North Carolina) described several such approaches. One involved a FRET-based biosensor for the active form of RhoA. This sensor was used to document activation of the protein at the front end of migrating cells, in contrast to the established view that RhoA activity is concentrated at the trailing end. Hahn also described a fluorescent probe for assessing Cdc42 activity in extending lamellipodia, and discussed the idea of introducing dyes into cells using nanoparticle cages. Gaudenz Danuser (Scripps Research Institute) described the application of “speckle” microscopy to the analysis of actin dynamics in living cells. Labeled actin monomers are introduced into cells at low molar ratios. Small clusters of labeled subunits in actin filaments form speckles whose movement is used to study flow of the actin network and whose appearance and disappearance are used to track rates of polymerization and depolymerization. Maps of these events are then correlated with cell movement and cytoplasmic extension to better understand the control of these processes. Tony Yeung (Hospital for Sick Children, Toronto) presented the use of fluorescently labeled polycationic probes to study changes in surface charge of the inner plasma membrane leaflet. This charge, which reflects the concentration of anionic phospholipids, was shown to disappear from membrane of internalized phagosomes. The probes should also be useful reporters of the movement of signaling proteins such as Src and Ras, which interact similarly with the membrane.

The symposium also included more systemic investigations of eukaryotic cell migration. Carole Parent and Annarita Bagorda (National Institutes of Health) described a “signal relay” system underlying the concerted cAMP-driven migration of Dictyostelium in long columns of cells. This is part of the aggregation response of the organism that occurs when the nutrient supply is low. They found that binding of extracellular cAMP to receptors at the leading edge of the cell, in addition to triggering the signals for chemotaxis toward the attractant, can also lead to activation of adenylate cyclase, expressed mainly at the trailing edge. Using a FRET-based cAMP sensor, they showed intracellular cAMP increases in response to a chemotaxtactant and proposed that it is subsequently secreted from the rear.
of the cell and serves as an attractant for the cell moving behind it. The mechanism of secretion is not well understood. Anna Huttenlocher (University of Wisconsin) reported studies of chemotaxis in vivo using a zebrafish model. Neutrophils labeled with GFP could be tracked migrating toward a wound at the edge of a fin. Cells leaving the vasculature moved toward the wound in highly directed trajectories at a speed of $\sim 11 \mu m/min$. Remarkably, cells could also be observed moving in the opposite direction with similar trajectories and speeds. This process, the basis of which is not yet clear, may be an important aspect of the resolution of an inflammatory response.

III. Phagocytosis

The process of phagocytosis comprised a third major topic of the symposium. This function shares many features in common with eukaryotic chemotaxis. It is receptor mediated, signals largely through small G proteins, and involves the directed remodeling of the actin cytoskeleton. Other aspects, such as the fusion of membrane processes around the particle being ingested, are unique to phagocytosis.

The basic events underlying phagocytosis were reviewed by Joel Swanson (University of Michigan) and by Steven Greenberg (Columbia University) (see Fig. 3). Many events begin with the activation of Fc receptors through binding of an IgG-coated particle. The receptors in turn assemble a group of proteins via an associated immunoreceptor tyrosine-based activation motif (ITAM). This leads to the activation of Src-family kinases that in turn activate Syk kinase and PI3 kinase. Downstream targets of these events include the small G proteins Cdc42, Rac1 and 2, and Arf6, which in turn coordinate actin polymerization, myosin regulation, and stimulation of the NADPH oxidase. The net result is the spreading of pseudopods around the target particle, producing additional interactions between receptors and ligands on the particle surface in a zipper-like process. Eventually, the pseudopods encircle the particle, leading ultimately to its internalization and digestion. The process is modulated by another set of inhibitory Fcγ receptors that couple to inhibitory mediators through associated immunoreceptor tyrosine-based inhibitory motifs (ITIM’s).

Swanson and Adam Hoppe (University of Michigan) emphasized the crucial aspects of timing of these responses. The various events are coordinated at least in part by phospholipid metabolism. Recruitment of PI3 kinase to the membrane phosphorylates PI(4,5)P₂ lipid to produce PIP₃ which activates a second pathway. PIP₃ is dephosphorylated back to PI(4,5)P₂ by PTEN (see above) but also by the 5’-phosphatase SHIP-1, producing PI(3,4)P₂ which has its own set of downstream targets. If PI3 kinase is inhibited, the phagocytic cup begins to form but the process stalls before the engulfment stage is completed. Under these conditions, Cdc42, Rac1, and Arf6 are activated but do not inactivate, while Rac2 fails to activate. Greenberg pointed out that overexpression of SHIP-1 blocks the phagocytosis of large but not of small particles, indicating that early events are sufficient if the target is not too big.
In addition to these canonical constituents of the signaling pathway, a number of additional proteins were shown to play a role in the phagocytosis process. Swanson showed that Arf1, a protein normally associated with the Golgi apparatus, is activated at the advancing edge of the phagocytic cup. This is a relatively late event and is also blocked by inhibition of PI3 kinase. Greenberg suggested a role for the adaptor protein Cbl, a substrate for Src tyrosine kinase that can also interact with Arp2/3, which in turn regulates actin polymerization. Cbl-b knockout cells show increased binding and phagocytosis. William Trimble (Hospital for Sick Children, Toronto) discussed the involvement of coronins, proteins known to associate with actin. He showed that coronin-1 associates with the phagosome, dropping off after internalization. A dominant-negative form of the protein inhibits phagocytosis and actin polymerization but not NADPH oxidase activation. This suggests that coronins could participate in early stages of the process, perhaps linking Arp2/3 with actin at critical sites where remodeling of the cytoskeleton is needed. Emmanuelle Caron (Imperial College, London) advocated a role for talin, a large cytoskeletal protein, in phagocytosis. Talin associates with the phagosome and its depletion by genetic knockout or RNAi leads to inhibition of the process.

Phagocytosis can also be driven through activation of complement receptors (CR3). Although the mechanism is not as well studied as that involving Fcy receptors, it is clear that somewhat different signaling pathways are involved. Gabriela Cassio (Hospital for Sick Children, Toronto) described some of the CR3-dependent events. These include the recruitment of Rac1 but not Rac2 or Cdc42 to the phagocytic cup. An interesting nuance is the appearance of two waves of actin polymerization. The first coincided with the closure of the phagosome, while the second occurred after the phagosome was completely internalized and preceded the appearance of an actin “comet tail.” This second wave may be involved in the movement of the organelle within the cell.

Invasion of tissues by metastatic cells involves the formation of cytoplasmic processes called invadopodia. The extension of these processes uses some of the same basic mechanisms as those of chemotaxis and phagocytosis. In addition, as described by Philippe Chavrier (CNRS/Institut Curie, Paris) the invadopodia must include membrane-tethered metalloproteases on their surfaces. He reported the involvement of Arf6 in this process. Arf6 helps to control the trafficking of recycling vesicles through interactions with the exocyst complex. Chavrier also discussed another crucial protein called IQGAP, which appears to connect actin with microtubules. The protein colocalizes with actin in the invadopodia and knockdown of its activity inhibits matrix degradation by tumor cells.

Bacterial invasion of mammalian cells is another phenomenon closely related to phagocytosis. In these cases, the invading organisms need to trick the host cells into the initiation of the internalization process. Two examples of such strategies were presented at the symposium.

Jorge Galán (Yale University) described the entry of Salmonella into epithelial cells. These cells do not normally carry out phagocytosis and lack expression of some of the proteins required for it. The bacteria get around this problem by supplying the proteins themselves. For this purpose they construct an injection device, designated a Type III secretion system, consisting of a tube that connects the cytoplasm of the bacterium and that of the host cell, and a motor for driving the transport of protein through the tube. The motor or injectosome is quite similar to that which is used by E. coli to force flagellin subunits into the central channel in order to elongate the flagellum (see above). The injected proteins include SopE and SopE2, both of which function as GEF’s that can activate Cdc42 and Rac. A third protein, SopB, has phosphoinositide phosphatase activity and can activate RhoG. These proteins work in concert to stimulate the uptake of the bacteria into the cells by a process that mimics phagocytosis.

A second, very different strategy used by Listeria was discussed by Keith Ireton (University of Central Florida). Here the bacterium expresses a protein called InIB on its surface that can mimic the ability of hepatocyte growth factor (HGF) to bind to and activate its receptor. InIB and HGF are not homologous or structurally related and do not compete for binding to the receptor. However, InIB–HGF receptor interaction recapitulates the major effects of HGF, including increased cell motility, actin remodeling, and eventually the internalization of the receptor through a clathrin-mediated process. Thus the bacterium makes use of the natural receptor signaling mechanism to make its way into the cytoplasm.

Summary
The meeting illustrated spectacular progress in identifying the molecular components underlying these three important biological processes. Future research will be directed toward a better understanding of the integration of these components to produce the complex behavioral responses. This work will include determination of structures of protein complexes and elucidation of the dynamics of macromolecular assemblies.

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