provided by STM spectroscopy. After comparing the STM atomic images at various bias voltages on the edge plane of the layer structure, Hasegawa *et al.* claimed that the BiO layer in Bi2212 (*16*) is insulative with gap energy several tens of millielectron volts or larger. This finding was contrary to the conclusions indicated by the band calculations and photoelectron spectroscopy. However, the insulative nature of the BiO layer has gradually been established by the observation of the S-I-S (superconductor-insulator-superconductor) type Josephson junction characteristics in the built-in layer structure (*17*) and by the most recent electron spectroscopy results (*18*). The CuO chain in YBCO was also found by the same method to exhibit a much lower density of states near the Fermi level (*19*).

Furthermore, the CuO chain, which was once believed to be the highly conductive path that gives the conductance anisotropy in the basal plane in YBCO, may form a charge density wave (CDW) with a wavelength several times the bond length of CuO (*20*). Such a wave is suggested by comparison of atomic images taken at different bias voltages, which yield the alternative appearance and disappearance of the CuO chain image along the crest and trough of a CDW on reversal of the sign of the bias voltage.

Thus, atomic site tunneling spectroscopy has emerged as a potential tool with which we can obtain excitation spectra of electrons as a function of atomic location—a new concept in solid-state physics.

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Checkpoints Take the Next Step

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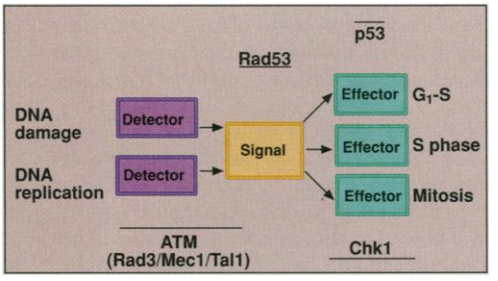
Treatment of dividing cells with radiation causes a pause in the G_2 phase of the cell cycle that stops the cell from proceeding through mitosis. When the pause is absent, the cells are more sensitive to radiation (*1*). These observations led to the concept of "checkpoints"—specific times within the cell cycle during which progression through the cycle can be delayed in response to either DNA damage or to incompletion of prior cell cycle events such as DNA replication. Several checkpoints monitor the integrity of the DNA: Radiation damage to DNA can delay the start of a new cell cycle (G_1-S checkpoint), DNA damage delays DNA synthesis (S phase progression checkpoint), DNA damage delays mitosis (DNA damage checkpoint), and errors in, or delays to, DNA synthesis delay mitosis (S-M checkpoint). Many proteins of the checkpoint pathways have been identified by genetic analysis of yeast (*2*), but the corresponding biochemical changes associated with their activation have not been analyzed. Two reports in this issue have now initiated the biochemical study of mitotic checkpoint pathways in yeast (*3*, *4*).

The Chk1 protein of fission yeast, a putative kinase, is required for mitotic arrest after DNA damage, but not for mitotic arrest when DNA synthesis is inhibited (*5*).



Checkpoint pathways: a detector, signal, and effector. The ATM family of proteins is probably part of the detector of DNA damage that stops cell cycle progression, whereas the Rad53 protein may be part of the signal pathways that activate the effectors such as Chk1 (mitosis) and possibly p53 (G_1-S transition).

Walworth and Bernards (*3*) have now demonstrated that Chk1 is phosphorylated in response to DNA damage but not when the S phase is inhibited by hydroxyurea, thus correlating the activation of a checkpoint pathway to a biochemical modification of a checkpoint protein. Mitotic checkpoints require three distinct functions: a detection system to determine the change in DNA structure; a signal pathway to transmit this information; and an effector mechanism to interact with the cell cycle machinery (see the figure). Genetic analysis of *chk1* mutants suggested that Chk1 functions as part of the effector mechanism, close to the cell cycle machinery (*5*, *6*). By examining the phosphorylation status of Chk1 after irradiation of various checkpoint mutants, Walworth and Bernards have formally placed Chk1 downstream of the majority of the fission yeast checkpoint proteins, consistent with such a role.

One of the checkpoint proteins upstream of Chk1 is Rad3, a large member of the phosphatidylinositol-3′ subgroup of kinases (*7*). The *rad3* protein shares significant amino acid homology with the human ATM protein, which is mutated in patients with the cancer-prone genetic disorder ataxia telangiectasia (*8*). Cells from patients with this disease show a phenotypic overlap with *rad3* mutants, suggesting functional as well as structural conservation. *Saccharomyces cerevisiae* has two gene products that are related to the ATM protein: Mec1, an essential protein required for the mitotic checkpoints and the homolog of *Schizosaccharomyces pombe* Rad3; and Tel1, which is involved in telomere maintenance. Although Tel1 mutants are not defective in checkpoints, Mec1 and Tel1 share some overlapping functions (*4*, *9*). Sanchez and co-workers (*4*) have now shown that the *RAD53* gene can act as a multicopy suppressor of *mec1*$^-$ lethality. Rad53 is required for DNA damage checkpoints and for the S phase–mitosis checkpoint, and it is also needed for the transcriptional response to DNA damage. In their re-

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port, Sanchez *et al.* demonstrate that the damage-dependent phosphorylation changes to Rad53 are lost in *mec1* mutant cells and can be restored by overexpression of the functionally overlapping *TEL1* gene (*4*).

In mammalian cells, a similar analysis of the stabilization of p53 (a biochemical marker for the G_1-S checkpoint) has indicated that the ATM protein functions in either a detector or signaling pathway that is upstream of the p53 effector for the G_1-S checkpoint (*10*). Furthermore, the involvement of ATM but not p53 in the G_2-M checkpoint indicates that the ATM pathway sends signals to distinct effectors for each cell cycle transition. Walworth and Bernards' data indicate that Chk1 is a cell cycle transition–specific effector at mitosis for the Rad3-dependent DNA damage checkpoint pathway. The data of Sanchez *et al.* show that Rad53, which has a more pleiotropic influence on several checkpoints, probably acts downstream of Mec1 and Tel1 in a pathway that signals to the different cell cycle transition–specific effectors such as Chk1. No checkpoint genes are identified as lying downstream of Chk1, consistent with its role as an effector. However, the data of Sanchez *et al.* suggest that *S. cerevisiae* Rad9 may be downstream of Rad53 because Rad9 does not appear to influence Rad53 phosphorylation. This result amply demonstrates the utility of a biochemical assay for the checkpoints because Rad9, along with Rad17 and Rad24, is involved in the processing of single-strand breaks into regions of single-stranded DNA. This property of Rad9 led to the suggestion that it is involved in the initiation of the checkpoint signal (*11*). But the new data suggest that the relation between checkpoint signal initiation and lesion processing may be more complicated.

The *ATM* gene encodes a human checkpoint protein related to Rad3 and Mec1; it is likely that human homologs of Chk1 and Rad53 also exist and that their activity will be influenced by the ATM pathway. The identification of a biochemical marker for checkpoints by Walworth and Bernards (*3*) and Sanchez *et al.* (*4*) will allow the analysis of the DNA structure checkpoints and their upstream activators in human cells, where genetic approaches are not available.

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Cellular Microbiology Emerging

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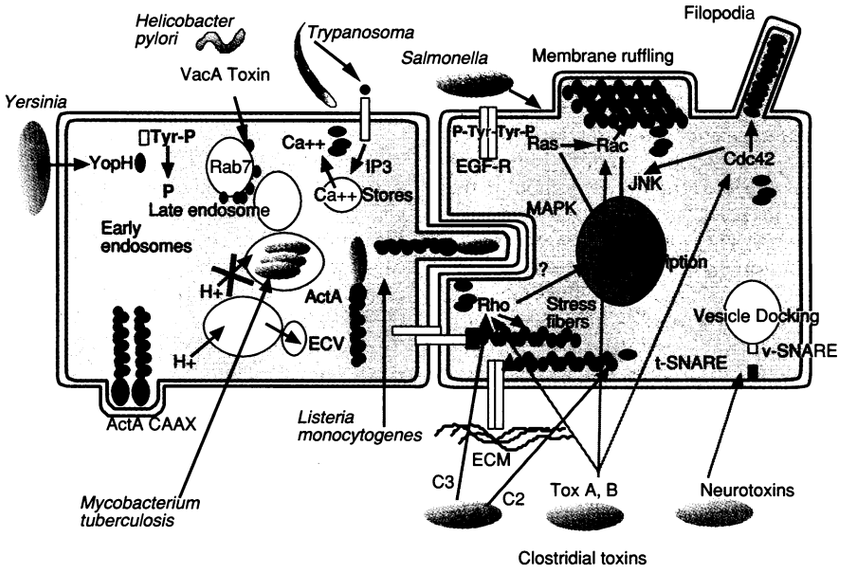
A new discipline, cellular microbiology, is emerging at the interface between cell biology and microbiology. Traditional cell biological approaches are already widely used to unravel the tactics microbes utilize to infect their hosts, but the use of pathogens to tackle questions in cell biology is just now yielding promising approaches and elegant results. Two meetings, in 1989 and 1991 (*1*), laid the groundwork for the field, and a third meeting in 1995 highlighted recent progress (*2*).

A major focus of this new field is the actin network, which together with intermediate filaments and microtubules constitute the cytoskeleton. The rapid assembly and disassembly of actin microfilaments is essential for phagocytosis, motility, cell division, and adhesion to a substratum or to another cell. Yet, the signaling pathways that control actin dynamics are poorly understood. Bacteria that can be genetically manipulated and parasites can provide tools to dissect these control pathways. When certain bacteria, such as *Salmonella* and *Shigella*, infect cells, they mimic the action of epidermal growth factor (EGF), inducing membrane ruffling and active actin polymerization (*3*–*5*) (see figure). The ruffling leads to internalization of the bacteria.

The internalization of other pathogens occurs without membrane ruffling or even actin polymerization. The parasite *Trypanosoma cruzi* enters cells by triggering a combination of events—a transient increase in cytosolic free calcium, rapid rearrangement of the cortical actin cytoskeleton, and lysosome recruitment and clustering at the invasion site (*6*, *7*). Lysosomes contribute membrane for the formation of the parasitophorous vacuole. Disruption of cortical actin by the increase in local calcium allows lysosomes to migrate and fuse, a phenomenon also regulated by calcium. Phospholipase C



Pathogenic bacteria interfere with numerous eukaryotic cell functions, providing a sophisticated tool kit for cell biologists.

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