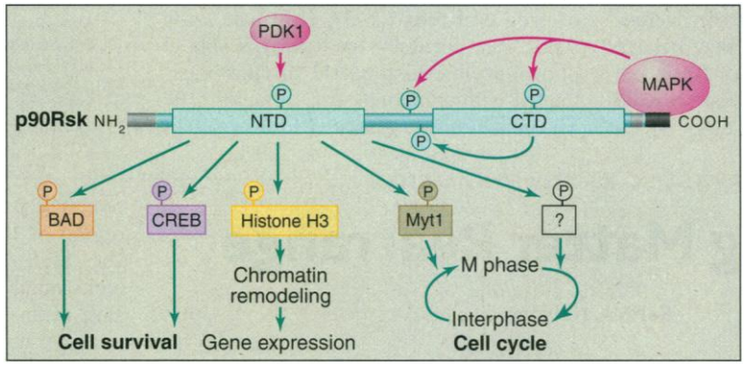
PERSPECTIVES: SIGNAL TRANSDUCTION

Cell Survival Demands Some Rsk

Angel R. Nebreda and Anne-Claude Gavin

The signals that regulate cell division, differentiation and death are initiated at the plasma membrane—for example, through the binding of a ligand to its receptor—and must somehow be transmitted to target molecules within the cell interior. The addition of phosphate groups to proteins (phosphorylation), is one important way in which signals from the cell surface are transmitted within the cell. A family of serine-threonine protein kinases called MAPKs (mitogen-activated protein kinases) that phosphorylate many cytoplasmic and nuclear target proteins are crucial regulators of the cellular responses induced by many extracellular stimuli (both mitogenic and nonmitogenic). For example, MAPKs directly phosphorylate and activate a number of transcription factors. In addition, they behave as cytoplasmic relay molecules by regulating downstream signaling proteins, including several other protein kinases. These pathways allow enzymatic amplification of the signal and provide a way for different signals from other pathways to be integrated. However, the importance of the signaling proteins downstream of the MAPKs is still not fully understood. Now, three reports in this issue on pages 1358, 1362, and 1365, respectively (*1*–*3*), show that cell survival and cell cycle regulation by a MAPK signaling pathway (the p42/p44 ERK pathway) involves the pp90 ribosomal S6 kinases (Rsks) as crucial downstream effector molecules.

Rsks (also referred to as MAPKAP kinases-1) are a family of 85- to 90-kD proteins that are widely expressed in higher eukaryotes. In yeast, Rsk-related protein kinases have not been identified so far. Mammals have at least three Rsk isoforms—Rsk-1, Rsk-2, and Rsk-3—that are all specifically activated through phosphorylation by ERK MAPKs but not by other MAPK subfamilies. Mutations in human Rsk-2 are associated with Coffin-Lowry syndrome, an X-linked disorder characterized by mental retardation and skeletal abnormalities. Rsks are unusual protein kinases in that they contain two kinase domains in a single polypeptide (see the figure). The amino-terminal domain of Rsk is required for phosphorylation of substrates such as the transcription factors CREB and c-fos and the protein kinase Myt1. The carboxyl-terminal domain is able to phosphorylate the linker sequence between the two kinase domains and in this way is thought to regulate the activity of the amino terminus. Rsks also contain an α helix downstream of the carboxyl-terminal domain, which upon deletion or mutation results in a permanently activated protein kinase (*4*). The phosphorylation and activation of Rsks by the ERK subfamily of MAPKs is dependent on the presence of a MAPK docking site at the carboxyl terminus (see the figure) (*5*, *6*). Although phosphorylation by MAPKs is essential, complete Rsk activation also requires phosphorylation of the amino-terminal domain by PDK1 (phosphoinositide-dependent kinase 1) (*7*, *8*). Thus, Rsk activation integrates regulatory inputs from both the MAPK- and PDK1-dependent signaling pathways. Curiously, the results reported by Gross *et al.* (*2*) point to an additional inhibitory role for the amino-terminal 43 amino acids of Rsks, suggesting that there may be additional mechanisms of Rsk regulation.



Risky business. Rsks are key molecules in the MAPK signal transduction pathway. They have an NH_2-terminal (NTD) and COOH-terminal kinase domain (CTD). Complete activation of Rsks requires that they be phosphorylated by MAPK and PDK1. Activation of Rsks also requires that they be autophosphorylated by their own CTD. The docking site for MAPK (black box) and the putative autoinhibitory domains (gray boxes) of Rsks are indicated. Rsks can phosphorylate a growing list of substrates involved in various cellular processes such as BAD in cell survival, histone H3 in chromatin remodeling, and Myt1 in cell cycle regulation.

It has been proposed that Rsks are important intermediates that connect MAPK activity with the transcriptional activation of key regulatory genes. These effects are mostly mediated by the direct association of Rsks with numerous transcription factors, which they phosphorylate and activate (*9*). Recently, a more general role for Rsks in transcriptional regulation has been postulated with the observation that Rsk-2 can phosphorylate histone H3, which may be important in chromatin remodeling (*10*). Rsks may also be involved in functions other than transcriptional activation, such as regulation of the cell cycle. In frog oocytes, Rsks phosphorylate the $p34^{cdc2}$ inhibitory kinase Myt1, decreasing its activity; this results in progression of oocytes through the G_2/M phase of meiosis (*11*). The down-regulation of $p34^{cdc2}$ inhibitory kinases by Rsks may also be important for progression of mammalian somatic cells through the G_2/M phase of mitosis (*12*).

The three reports in this issue provide strong support for Rsk's involvement in new and rather unexpected activities. Bonni *et al.* (*1*) report that Rsk-2 is involved in the survival of cerebellar neurons that have been treated with a survival growth factor. They found that Rsk-2 phosphorylates and inactivates the pro-apoptotic protein BAD, a component of the cell death machinery. The protein kinase Akt is also able to phosphorylate and inhibit BAD. Thus, MAPK/Rsk-2 and the phosphoinositide 3-kinase/Akt signaling pathways converge at the level of BAD inactivation to promote the survival of cells treated with survival growth factors. Interestingly, besides the inactivation of BAD, Rsk-2 is probably also involved in the transcriptional up-regulation of the pro-survival gene *Bcl-2* through the phosphorylation and activation of the transcription factor CREB. This demonstrates that Rsk-2 can relay both transcription-dependent and transcription-independent signals through the MAPK signal transduction pathway within the same cell.

Two additional reports demonstrate that Rsks are also involved in MAPK-mediated arrest in the metaphase II stage of meiosis (*2*, *3*). In most vertebrate species the meiotic cell cycle is blocked in metaphase II by a cytoplasmic activity called cytostatic factor. The biochemical nature of this factor has not yet been elucidated, but it is known that

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the activation of MAPK by the protein kinase Mos is essential for its activity (*13*). Bhatt and Ferrell (*3*) now show that the metaphase arrest induced by the activation of the Mos/MAPK cascade in cell-free extracts prepared from frog embryos can be abrogated by the specific immunodepletion of Rsk-2 from the extracts. Interestingly, adding back physiological concentrations of recombinant Rsk-2 protein restores the ability of the extract to undergo metaphase arrest in response to Mos. These results are complemented in the accompanying report by Gross *et al.* (*2*), who show that a constitutively activated form of Rsk-1 can induce metaphase arrest in cleaving frog embryos in the absence of any detectable endogenous MAPK activity. These findings raise the intriguing possibility that Rsks may be the only MAPK substrate required for cytostatic factor activity. However, it is also possible that the truncated Rsks that were overexpressed in these experiments may behave nonphysiologically. The work of Bhatt and Ferrell (*3*) further suggests that Rsks may be responsible for some of the changes in cell morphology that occur during cell division. This begs the question of what the Rsks are actually doing. Although the protein targets of Rsks remain unclear, a promising candidate is the ubiquitin-dependent proteolysis machinery that plays an important role in cell cycle regulation.

To understand the mechanism responsible for metaphase II arrest, the new studies suggest that future work should focus on finding the targets of Rsk rather than those of MAPK. The same may be true for other cellular processes involving the activation of MAPKs. In this regard, development of antibodies specific for known Rsk phosphorylation motifs (*4*) will be useful for identifying new Rsk substrates. It is still not clear whether different Rsk isoforms have different functions and protein targets. Both Rsk-1 and Rsk-2 appear to have similar effects on the metaphase II arrest of frog embryos (*2*, *3*). However, only Rsk-2 (but not Rsk-1 or Rsk-3) is able to phosphorylate histone H3 in fibroblasts treated with epidermal growth factor (*10*). The constitutively activated forms of Rsks described in this issue (*2*) and elsewhere (*4*) should be of great help in investigating new Rsk functions. A rewarding task for the future will be to generate reagents to specifically inhibit Rsk signaling, allowing the contribution of Rsks to various cellular responses to be deciphered. As the three new studies suggest, such an approach could reveal an even more important job for Rsks in the MAPK signaling pathway than previously thought.

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PERSPECTIVES: ULTRAFAST X-RAY DIFFRACTION

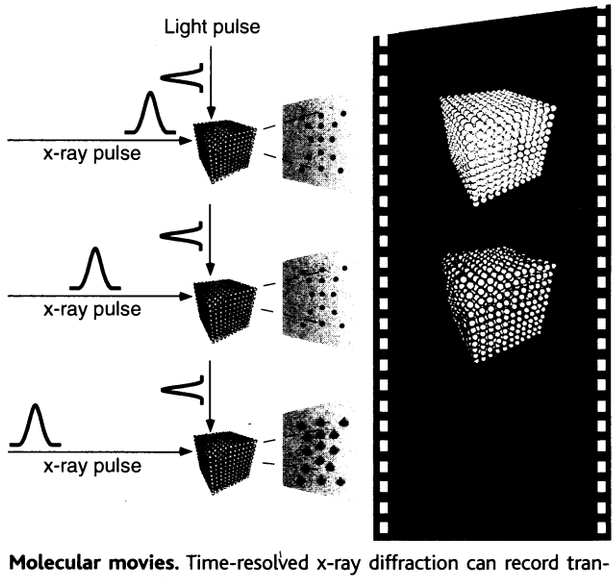
Watching Matter Rearrange

Keith A. Nelson

Can we "watch" the elementary molecular or collective motions that lead to chemical or structural change as they take place (see the figure below)? On page 1340 of this issue, Siders *et al.* (*1*) report an important step toward this goal. Using ultrafast pulses of x-ray radiation as the probe, they monitor the melting of a semiconductor sample after irradiation by an intense, ultrafast visible pulse. The experiment (see the figure on the next page) allows the direct measurement of the changes in crystal lattice structure and loss of crystalline order associated with melting. The results represent the early fruits of a worldwide effort to generate ultrashort hard x-ray pulses and use them for the characterization of ultrafast events through time-resolved x-ray diffraction (*2*–*5*).

In earlier work, the same group was able to monitor light-driven sound waves that give rise to time-dependent changes in unit cell volume (*6*). The next step, namely to watch the sample undergo collective structural change during a solid-liquid phase transition (*1*), introduced numerous experimental difficulties, not the least of which is that each laser shot destroys the irradiated region of the single-crystal thin-film sample. Still, enough data could be collected to reveal a precipitous drop in x-ray diffraction intensity within the first few picoseconds of intense optical excitation. The results indicate that the excited region of the sample, which contains a very high density of energetic electrons, melts quickly—long before the electrons have given up most of their energy to lattice vibrations—and homogeneously, in contrast to the more usual case (observed in less highly excited sample regions) of surface melting followed by propagation of the melt front into the sample at a fraction of the speed of sound.

Of course, we can already monitor ultrafast time-dependent motion in chemical reactions and structural rearrangements using femtosecond optical pulses (*7*). This year's Nobel Prize in Chemistry, awarded to Ahmed Zewail for the development of "femtochemistry," recognizes remarkable achievements in monitoring chemical bond breakage in photochemically reactive molecules (*8*). These events are monitored with light in the visible or nearby spectral regions, rather than x-rays, and the probe pulse therefore "watches" the valence electrons, rather than the positions of atomic nuclei. That is fine for



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Molecular movies. Time-resolved x-ray diffraction can record transient structures of materials as they undergo rapid change initiated by an ultrashort optical pulse.

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