grain boundaries results in straining of the rock. In contrast, anhydrite aggregates (*7*) decrease substantially in strength at a shear strain of about $\gamma = 1$. This drop in strength results from a change in deformation mechanism from dislocation creep to diffusional creep as the grain size of the rock is reduced by dynamic recrystallization. Marble and magnesiowüstite aggregates (*5*, *8*) show no change in deformation mechanism and little change in strength with increasing strain, despite recrystallization to a substantially finer grain size. After reaching a peak at modest strains, the strength of olivine aggregates (*1*) decreases as the grains recrystallize to a finer grain size. No change in deformation mechanism is observed.

All these rocks thus show substantial changes in microstructure and texture during deformation, with large reductions in grain size, but the deformation mechanism changes in only one of the materials (anhydrite). It should therefore not be assumed that recrystallization and associated grain-size reduction during high-strain deformation in Earth's interior will necessarily result in changes in deformation mechanism (*9*–*11*).

Many modeling studies of the deformation of Earth's upper mantle use the rheological data of Karato *et al.* (*10*), which were collected during low-strain deformation experiments of olivine aggregates in both dislocation and diffusional creep fields. No changes in deformation mechanism due to dynamic recrystallization were observed experimentally, but these results have nevertheless been used to predict mechanism changes due to recrystallization as a function of depth in the upper mantle (*11*).

The new results (*1*), albeit collected over a limited range of experimental conditions, show no such changes in deformation mechanism for olivine-dominated rocks despite substantial grain-size reduction. Ultimate resolution of the question as to whether grain-size reduction by dynamic recrystallization changes the deformation mechanism in the upper mantle must await further experimental data covering a broader range of conditions. Until then, we should be careful not to invoke deformation mechanisms for which there is no definitive evidence.

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PERSPECTIVES: SIGNAL TRANSDUCTION

An Arresting Start for MAPK

Jacques Pouysségur

Protein kinases are ubiquitous enzymes that are able to modulate the activities of other proteins by adding phosphate groups to their tyrosine, serine, or threonine amino acids (phosphorylation). Mitogen-activated protein kinases (MAPKs), which are activated by many different signals, belong to a large family of serine/threonine protein kinases that are conserved in organisms as diverse as yeast and humans. MAPKs deliver extracellular signals from activated receptors to various cellular compartments, notably the nucleus, where they direct the execution of appropriate genetic programs. A unique feature of MAPKs is that they themselves can be activated by addition of phosphate groups to both their tyrosine and threonine amino acids (dual phosphorylation) after stimulation of a receptor by growth factors, mitogens, hormones, cytokines, or environmental stresses. MAPKs operate in modules composed of three protein kinases that phosphorylate and activate each other sequentially: MAP kinase kinase kinase (MKKK) activates MAP kinase kinase (MKK), which then activates MAP kinase. These kinase modules have been duplicated with slight variations, allowing cells to instigate multiple biological responses through a set of MAP kinase–wiring networks.

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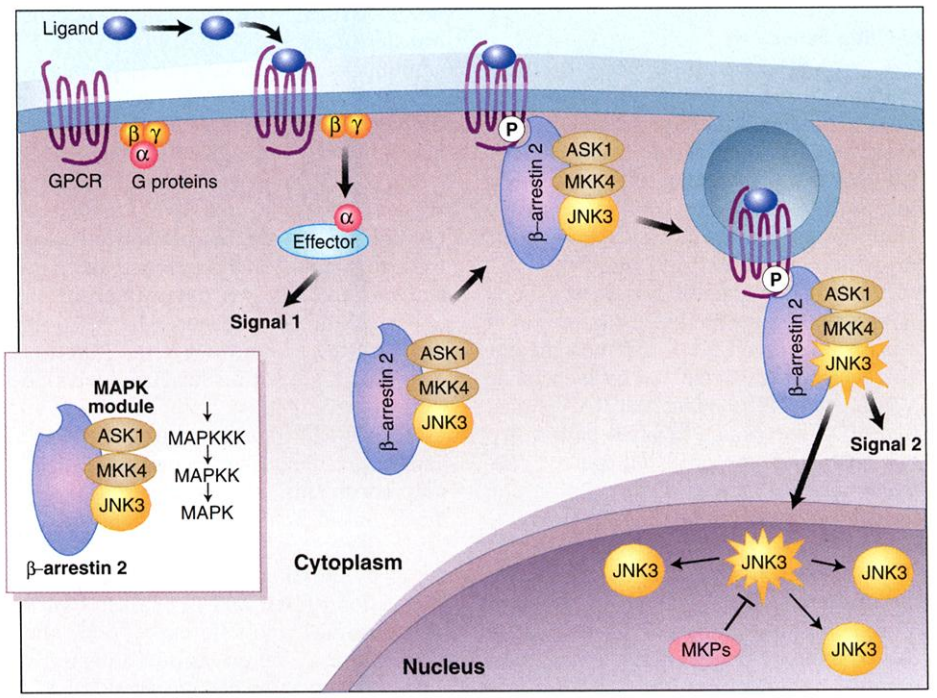
The coexistence of conserved protein kinase modules within the same cellular compartment, however, poses an enormous challenge for the cell because protein kinases are rather promiscuous enzymes. How does the cell solve the crucial problems of substrate specificity and the prevention of inappropriate cross talk between signaling pathways? How are these highly conserved signaling circuits "insulated" from nonspecific interactions with other molecules? Cells seem to have adopted two solutions. The specificity and fidelity of kinases is ensured by the existence of specific docking sites on kinases and their protein substrates (*1*, *2*). Insulation and signal efficiency are provided by scaffold proteins that assemble the components of a given MAPK module into a single signaling complex (*3*). Attempts to identify scaffold proteins have been frustrated by the fact that they are not enzymes and seem to have emerged as nonconserved evolutionary "bricolage." The first MAPK scaffold protein identified was Ste5 in budding yeast. An astonishing and intriguing new example of a mammalian scaffold protein is presented by McDonald *et al.* (*4*) on page 1574 of this issue. The investigators provide evidence that this new scaffold protein, β-arrestin 2, brings together components of the MAPK module, resulting in activation of c-Jun amino-terminal kinase–3 (JNK3) in response to activation of G protein–coupled receptors (GPCRs).

The β-arrestin 2 scaffold protein is not homologous to c-Jun amino-terminal kinase interacting protein (JIP), a member of the mammalian JNK scaffold protein family (*3*). This discovery is intriguing because for years it has been known that arrestins stop signals from growth factors, hormones, or environmental stressors by uncoupling the activated GPCR from its G protein signaling molecule (*5*, *6*). But how can arrestins be both "Stop" molecules and signal activators? What is the evidence that arrestins are scaffold proteins for the MAPK module that activates JNK3 through the activation of ASK1 and MKK4?

With a yeast two-hybrid screening assay, McDonald *et al.* identified a direct interaction between β-arrestin 2 and the carboxyl-terminal portion of JNK MAPK family members, in particular with the p54 splice variants of JNK. Immunoprecipitation of β-arrestin 1 and 2 from extracts of cultured cells expressing epitope-tagged JNK1, JNK2, and JNK3 isoforms established that only β-arrestin 2 coimmunoprecipitated with the JNK3 isoform. The authors then explored the functional consequence of this interaction on JNK3 activation by measuring phosphorylation of c-Jun, a typical transcription factor targeted by the JNK signaling pathway. JNK3 phosphorylated c-Jun only when it was coexpressed in cultured cells with both β-arrestin 2 and ASK1 (an upstream MKKK activator of the pathway). Because ASK1 cannot directly activate JNK3, this result suggested that MKK4 or MKK7 is present in the complex and that β-arrestin 2 may serve as a scaffold pro-

tein, holding together all of the components of the JNK cascade. ASK1 binds directly to the amino-terminal region of β-arrestin 2 at a site distinct from JNK3 binding. Whereas MKK4 or MKK7 displays a very weak interaction with β-arrestin 2, expression of ASK1 or JNK3 is sufficient to promote MKK4 recruitment to the complex. This finding suggests that MKK4 interacts indirectly with β-arrestin 2 through ASK1 or JNK3.

According to this evidence, the designation of β-arrestin 2 as a specific scaffold of β-arrestin 2 into these cells, angiotensin II initiated a time-dependent and robust activation of JNK3. Because no ASK1 was coexpressed in these cells, this finding implied that an endogenous MKKK was coopted into the complex. This was nicely confirmed by the finding that the three components of the MAPK module—ASK1, MKK4, and JNK3—were found in β-arrestin 2 immunoprecipitates; however, the amounts detected were not affected by addition of angiotensin II. This result suggests that the β-arrestin 2–JNK module is preformed in unstimulated cells and raises the question of how GPCR is involved in JNK3 activation.



β-arrestins court MAP kinases. Binding of a G protein–coupled receptor (GPCR) to its ligand activates a heterotrimeric G protein (Gαβγ). The activated Gα subunit interacts with an effector (for example, adenylyl cyclase, phosphodiesterase, or an ion channel) to deliver signal 1. The ligand-occupied receptor is then phosphorylated (P) by one of the GPCR-kinases, providing a binding site for a β-arrestin scaffolding protein. β-Arrestin then moves from the cytosol to the phosphorylated GPCR in the plasma membrane, thereby leading to G protein uncoupling and attenuation of signal 1. The complex between β-arrestin 2 and the JNK MAPK module (ASK1 activates MKK4, which activates JNK3) is constitutively preformed in the cytoplasm, and this entire complex translocates to the membrane-bound GPCR after ligand binding. β-Arrestin promotes internalization of the entire complex through clathrin-coated pits (*9*), leading to activation of the MAPK module and generation of signal 2. JNK3 is released from the β-arrestin 2 scaffold, translocates to the nucleus, and switches on transcription of target genes. JNK3 is then inactivated in turn by the nuclear MAPK phosphatases (MKPs) and is recycled back to the "activating" scaffold complex in the cytoplasm.

protein (*2*) is convincing. But still unanswered is the question of whether this β-arrestin 2–JNK3 complex is involved in GPCR-mediated activation of JNK3. The investigators addressed this question with cultured cells that expressed both JNK3 and a GPCR called angiotensin II receptor type IA. When the ligand for this receptor, angiotensin II, was added to the cells, there was a very weak and slow activation of JNK3. Remarkably, after introduction

A key feature of arrestins is their capacity to translocate from the cytosol to the vicinity of the phosphorylated GPCR in the plasma membrane, after activation of the receptor by an agonist (a molecule that resembles the receptor's natural ligand). Addition of angiotensin II to cells initiated a redistribution of intracellular β-arrestin 2 and JNK3 pools. A pool of β-arrestin 2 was found associated with endosomal vesicles, and part of the JNK3 pool, previously sequestered in the cytoplasm, moved to the nucleus. This nuclear JNK3 pool was, however, inactive. This intriguing finding hints that activation of the JNK3 module proceeds at the plasma membrane by recruitment of the β-arrestin 2–JNK3 complex to the activated GPCR (see the figure). Activated JNK3 is predicted to rapidly dissociate from the scaffolding ("activating") complex and to phosphorylate cytoplasmic and nuclear protein substrates. The inactive nuclear pool of JNK3 is reminiscent of the stimulation of p42/p44 MAPKs (also called extracellular signal–regulated kinases, or ERKs) by growth factors. In response to such stimulation, ERKs rapidly dissociate from the cytoplasmic activating complex. They then translocate to the nucleus where they accumulate and become progressively inactivated by nuclear MAPK phosphatases (which remove their phosphate groups).

The model that emerges from the McDonald study is one in which β-arrestin 2 is a dual signaling switch. Its translocation to the GPCR in the plasma membrane initiates the JNK3 signaling pathway, an action that simultaneously stops the G protein–mediated signal (see the figure). This appealing model immediately raises several questions: How is the arrestin–JNK module complex activated? Is the tethering of arrestin to the phosphorylated GPCR the initiating event? Is this multicomponent complex consisting of GPCR, β-arrestin 2, and the MAPK module formed in cells in vivo? Can we mimic JNK3 activation simply by inducing nonspecifically the translocation of β-arrestin 2 to the plasma membrane? How general is this signaling mechanism, and how important is it likely to be in cells that have not been artificially transfected with the components of this signaling pathway? Do all arrestin isoforms primarily serve as scaffold molecules for a subset of MAPK modules?

An emerging body of evidence lends strong support to the generality of this model. Several GPCRs, when activated by their appropriate ligands and engaged by β-arrestin 1 during endocytosis, have been found to be associated with the p42/p44 MAPK (ERK) module. The proteinase-activated receptor 2 (PAR2) complex contains the internalized receptor, β-arrestin 1, Raf-1, and p42/p44 MAPK (*7*). When activated under these conditions, p42/p44 MAPK does not translocate to the nucleus and, as expected, PAR2 does not transmit mitogenic signals inducing the cell to divide (*8*). Engineering mutations in PAR2 that inter-

fere with its interaction with β-arrestin prevents this receptor from becoming internalized by the cell and instead triggers activation of p42/p44 MAPK through a separate route, resulting in translocation of p42/p44 MAPK to the nucleus and mitogenic stimulation of the cell (*7*).

Several findings now converge to enlarge the part played by arrestins in signal termination. Receptor signaling and desensitization of the receptor to the activating signal are in reality two intimately coupled processes. Molecules viewed as "signal terminators" in one pathway may in fact be "activators" in another. With the McDonald *et al.* work, we are witnessing an important new beginning for arrestins as activators of MAPK signaling.

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PERSPECTIVES: GENETICS

Reprogramming X Inactivation

Philippe Clerc and Philip Avner

Several mammalian species including mouse (*1–3*), sheep, and pig have been successfully cloned by somatic cell nuclear transfer—that is, transfer of a nucleus from a differentiated adult cell into an oocyte that has had its own nucleus removed. Yet, despite these successes, few cloned embryos develop to term. Although the precise reasons for this are not clear, it is possible that the adult nucleus cannot be easily reprogrammed to direct embryonic development. On page 1578 of this issue, Eggan and colleagues (*4*) investigate how the transplanted nuclei of adult somatic cells and of embryonic stem cells are reprogrammed in cloned female mouse embryos. By monitoring X chromosome gene expression in different embryonic and extraembryonic (placental) tissues, the investigators were able to examine reprogramming of the X chromosome inactivation state of adult nuclei.

One of the two X chromosomes in female cells has to be shut down (transcriptionally silenced), otherwise twice the amount of protein will be produced in female cells as in male cells (which have only one X chromosome). There are several molecular processes that contribute to X chromosome inactivation [for a review, see (*5*)]. These include coating of the X chromosome by *Xist* nuclear RNA, removal of acetyl groups (hypoacetylation) from the histone proteins of DNA, accumulation of the macroH2A histone protein, and addition of methyl groups to CpG islands (stretches of cytosine and guanine nucleotides in the DNA). During the earliest phases of female embryogenesis, there is gene expression from both the maternal X chromosome (donated by the egg) and the paternal X chromosome donated by the sperm (see the figure, top). At the blastocyst stage, the X chromosome inherited from the father is preferentially chosen for inactivation in cells of the trophectoderm (the blastocyst outer layer). Later, when the embryo becomes implanted in the uterine wall, random X inactivation occurs in cells of the inner cell mass that will form all of the somatic tissues (*5*). Early inactivation events are under the control of a master region on the X chromosome, the Xic, which contains the *Xist* gene encoding a large nontranscribed nuclear RNA. Initiation of X inactivation involves both a step in which X chromosomes are counted relative to the total number of chromosomes in the cell and a process of choosing, whereby one of the two X chromosomes in the female cell may be preferentially selected for inactivation.

Eggan *et al.* studied X inactivation in cloned mouse embryos derived from enucleated oocytes injected with female adult cell nuclei (see the figure, bottom). In one set of experiments, the female adult nuclei transferred to oocytes had one X chromosome marked by a green fluorescent transgene (X^{GFP}) and the other marked by a null mutation in the *HPRT* gene. These cells are fluorescent when the transgene is carried by an active X chromosome (X_a) and is expressed, but are nonfluorescent when the transgene is carried by an inactivated X chromosome (X_i) and remains silent (*6*). Preparations of cells bearing an inactive X^{GFP} transgene ($X^{GFP}{}_i$) were obtained by selection with 6-thioguanine, which killed those cells expressing the normal *HPRT* gene; preparations of cells bearing an active X^{GFP} transgene ($X^{GFP}{}_a$) were obtained by selection in HAT medium, which killed those cells not expressing the *HPRT* gene. All blastocysts derived from [$X^{GFP}{}_i X_a$] nuclear transfer were fluorescent, supporting the conclusion that $X^{GFP}{}_i$ is reactivated in most cells of the preimplantation embryo. Reactivation of the X_i was followed by random inactivation of either X in the epiblastic cell lineages of cloned embryos (the epiblast, derived from the inner cell mass, will form all somatic tissues). This was known because transfer of either [$X^{GFP}{}_i X_a$] or [$X^{GFP}{}_a X_i$] adult nuclei resulted in a mixed population of cells bearing an $X^{GFP}{}_i$ and an $X^{GFP}{}_a$. Fluorescence-activated cell sorting of fibroblast cells derived from three mid-gestation E12.5 (embryonic day 12.5) clones revealed the $X^{GFP}{}_i$ and $X^{GFP}{}_a$ mixed cell populations. In extraembryonic tissues from four mid-gestation clones, the X inactivation status was determined by the donor cell nucleus because [$X^{GFP}{}_i X_a$] nuclei resulted in nonfluorescent placentas whereas [$X^{GFP}{}_a X_i$] nuclei generated fluorescent placentas.

The conclusions drawn from these elegant experiments were confirmed using polymorphisms (sequence variations) in the X-linked *Xist* and *Grpr* genes of embryos cloned from cells of F_1 females produced by crossing two mouse subspecies. Expression of *Xist* and *Grpr* from both X chromosomes (biallelic expression) confirmed that X reactivation followed by random inactivation had occurred in E13.5 tissues. In the placentas of six E13.5 cloned embryos, monoallelic expression of both *Xist* and *Grpr* was observed, which confirmed that X inactivation was nonrandom in extraembryonic tissues. Interestingly, transfer of nuclei from embryonic stem cells that have two active X chromosomes was associated with biallelic expression of *Xist* and *Grpr* in the placenta of the single recovered mid-gestation embryo. Thus, X inactivation in the extraembryonic territory of cloned embryos seems to occur randomly in the absence of the inactivation profile provided by the adult cell nucleus.

Given the extreme stability of X inactivation in adult tissues, it may seem remarkable that the early embryo can reverse the pattern of X inactivation (a process normally associated with formation of female gametes). It is possible, however, that the cloned embryo is mimicking properties of the natural embryo. During the early stages of normal embryogenesis, *Xist* RNA coats the paternal X chromosome (see the figure, top) (*7*). This

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