The two studies contribute new evidence of the effects of solar variability on climate (see the figure) and indicate avenues for further research into the mechanisms involved. It is clear that the complex links between the middle and lower atmospheres and between the atmosphere and oceans are key to a better understanding of these mechanisms.

References

1. G. Bond *et al.*, *Science* **294**, 2130 (2001); published online 15 November 2001 (10.1126/science.1065680).
2. J. Luterbacher *et al.*, *Geophys. Res. Lett.* **26**, 2745 (1999).
3. T. J. Osborn *et al.*, *Clim. Dyn.* **15**, 685 (1999).
4. M. P. Baldwin, T. J. Dunkerton, *Science* **294**, 581 (2001).
5. K. Kodera, *J. Geophys. Res.* **100**, 14077 (1995).
6. A. Robock, *Bull. Am. Meteorol. Soc.* **82**, 2189 (2001).
7. D. T. Shindell, G. A. Schmidt, M. E. Mann, D. Rind, A. Waple, *Science* **294**, 2149 (2001).
8. J. D. Haigh, *Science* **272**, 981 (1996).
9. _____, *Q. J. R. Meteorol. Soc.* **125**, 871 (1999).
10. A. Larkin *et al.*, *Space Sci. Rev.* **94**, 199 (2000).
11. D. T. Shindell *et al.*, *Science* **284**, 305 (1999).
12. H. van Loon, D. J. Shea, *Geophys. Res. Lett.* **26**, 2893 (1999).
13. M. Mann *et al.*, *Nature* **392**, 779 (1998).

PERSPECTIVES: SIGNAL TRANSDUCTION

RIPping Tyrosine Kinase Receptors Apart

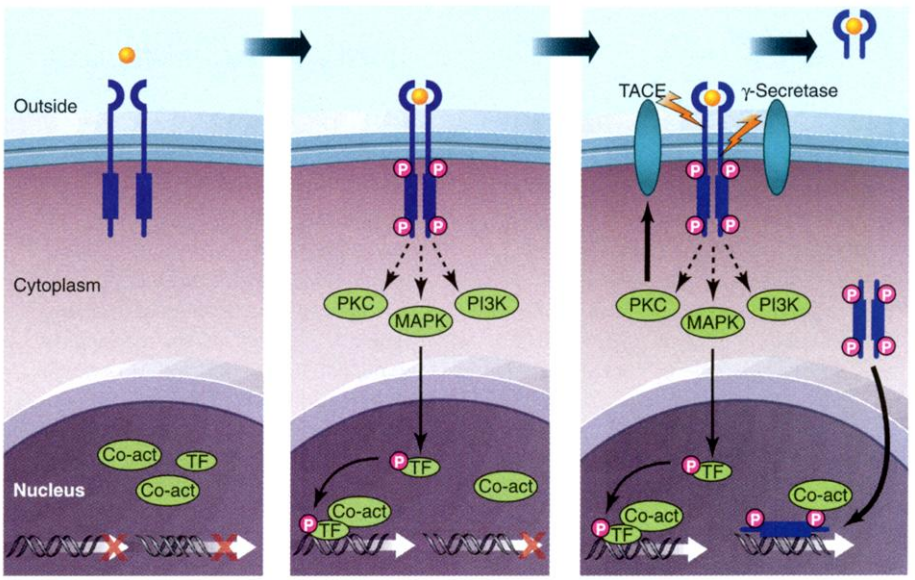
Carl-Henrik Heldin and Johan Ericsson

Tyrosine kinase receptors are transmembrane proteins that transduce signals controlling cell growth, survival, motility, and differentiation. These signals are activated by binding of a ligand (usually a growth factor) to the receptor, which then forms a dimer. This dimer adds phosphate groups to itself (autophosphorylation), resulting in the creation of docking sites that bind to downstream signal transduction molecules containing Src homology 2 (SH2) domains. Upon ligand binding, a number of signaling pathways can be activated, each one consisting of a chain of signaling molecules that indirectly alter the expression of target genes in the nucleus (see the figure). On page 2179 of this issue, Ni *et al.* (*1*) show that ErbB-4, a member of the epidermal growth factor (EGF) tyrosine kinase receptor family, also activates gene expression in a more direct manner. The ErbB-4 receptor undergoes proteolysis within its plasma membrane domain—a process called RIP for regulated intramembrane proteolysis—and its intracellular portion moves to the nucleus, where it may affect the transcription of target genes (see the figure).

There are two steps to the proteolysis of ErbB-4. First, most of this receptor's ectodomain is cleaved off by a membrane-associated metalloprotease called TACE. Next, the remaining part of the receptor is cleaved within its transmembrane domain by a second protease. This scenario is remarkably similar to the cleaving of certain other proteins that undergo RIP. These include Notch, a receptor involved in fate decisions during embryonic development; amyloid precursor protein (APP), a transmembrane protein of unknown function from which the extracellular β-amyloid peptide implicated in Alzheimer's disease is derived; and sterol regulatory element binding proteins (SREBPs), transmembrane proteins of the endoplasmic reticulum that regulate lipid metabolism (*2*). In all of these cases, most of the protein ectodomain is removed during the first cleavage. This triggers a second cleavage in the transmembrane domain, leading to the release of the cytoplasmic portion of the protein, which moves to the nucleus. Interestingly, the enzyme that catalyzes the secondary cleavage of ErbB-4, Notch, and APP is γ-secretase, also called presenilin (*3*). This γ-secretase is an aspartyl protease with the unusual ability to cleave proteins within their transmembrane domains. It spans the membrane several times and may form a channel-like pore in which proteolysis can occur.

There is firm evidence that the intracellular portions of Notch and SREBP modulate gene transcription (*2*). Notch interacts with the coactivator p300 (*4*), and SREBPs contain well-characterized DNA binding and transactivating domains that interact with a number of other transcription factors and coactivators (*5*). Furthermore, the intracellular portion of APP forms a complex with the nuclear adapter protein Fe65 and with Tip60 (which has histone acetyltransferase activity like p300), and stimulates transcription when fused to the DNA binding domains of the heterologous transcription factors Gal4 or LexA (*6, 7*). The important question provoked by the Ni *et al.* findings (*1*) is whether the intracellular region of cleaved ErbB-4 is able to affect gene expression in the nucleus.

Ni and colleagues found that blocking γ-secretase activity overcame the ligand-induced growth inhibition of fibroblasts overexpressing ErbB-4. This suggests that release of the intracellular portion of the



Regulated intramembrane proteolysis of a tyrosine kinase receptor. Binding of a ligand to its tyrosine kinase receptor induces activation and autophosphorylation of the receptor and the creation of docking sites for signaling proteins containing SH2 domains. In this way, different signaling pathways, such as that containing mitogen-activated protein (MAP) kinase, are activated. In a separate signaling pathway, PLC-γ activates PKC, which then activates the metalloprotease TACE. This enzyme cleaves off the ectodomain of the receptor and allows intramembrane cleavage of the remaining part by γ-secretase. The cleaved cytoplasmic region of the receptor then moves to the nucleus, where it may affect the transcription of target genes.

The authors are at the Ludwig Institute for Cancer Research, Box 595, Biomedical Center, SE-751 24 Uppsala, Sweden.

ErbB-4 receptor is important for expression of genes controlling growth inhibition. Ni *et al.* also report that the carboxyl-terminal region of ErbB-4 has weak transcriptional activity when fused to the DNA binding domain of GAL4. Interestingly, Lin *et al.* (*8*) recently reported that the EGF receptor, a relative of ErbB-4, may also act directly in the nucleus and may affect gene expression. They found that a fraction of the EGF receptor moved to the nucleus after activation of the receptor by ligand binding. A strong transcriptional activity was observed when the carboxyl-terminal region of the EGF receptor was fused to the DNA binding domain of Gal4. Furthermore, the EGF receptor complex bound to an AT-rich DNA sequence motif. The promoter for the gene encoding cyclin D1 contains two copies of this AT-rich motif, which are required for activation of this gene by EGF. Together, the results of Ni *et al.* (*1*) and Lin *et al.* (*8*) suggest that cytoplasmic fragments of members of the EGF receptor family are important regulators of gene expression.

A crucial question is how receptor cleavage is itself regulated. It appears that, for the cases studied so far, the triggering event is the removal of most of the extracytoplasmic part of the protein. This appears to be a prerequisite for intramembrane cleavage by γ-secretase or related proteases. In the case of ErbB-4, the metalloprotease TACE that removes its ectodomain is activated by protein kinase C (PKC). The physiological activator of PKC is phospholipase, but experimentally, as used by Ni *et al.* (*1*), the phorbol ester TPA also does the job. Interestingly, phospholipase C–γ is one of the SH2 domain proteins that are activated by several tyrosine kinase receptors. Thus, intramembrane proteolysis of ErbB-4 may be induced by its activation of SH2 domain proteins (see the figure).

It is still unclear how the liberated intracellular fragments of the receptors are translocated to the nucleus. Because only a small portion of ErbB-4 undergoes RIP, it is likely that nuclear transport does not occur by random diffusion but rather is carefully regulated. Ni *et al.* report that ErbB-4 has putative nuclear localization and nuclear export sequences. At the very least, the nuclear export sequence is involved in nuclear shuttling of the receptor fragment, because accumulation of the ErbB-4 receptor fragment in the nucleus is enhanced by leptomycin B, which blocks nuclear export of proteins (*1*). Lin *et al.* (*8*) also identified a putative nuclear localization sequence in the juxtamembrane portion of the EGF receptor. This sequence is conserved in all members of the EGF receptor family.

In both ErbB-4 and the EGF receptor, it is the extreme carboxyl-terminal region of the receptor that is the strongest activator of transcription. However, the entire cytoplasmic domain of both receptors appears to be translocated to the nucleus. It is possible that additional specific proteolytic events take place in the nucleus such that the juxtamembrane domain (containing the nuclear localization signal) and the kinase domain are cleaved off, liberating the transcriptionally active carboxyl-terminal tail. Another intriguing possibility is that the cytoplasmic receptor domains in the nucleus also regulate transcription through phosphorylation events.

The discovery that RIP is another way in which the cell conveys signals from the plasma membrane (and endoplasmic reticulum membrane) to the nucleus provides a new example of the multifaceted nature of intracellular signal transduction. The Ni *et al.* work shows that tyrosine kinase receptors are also subject to RIP. One avenue for future investigation will be to elucidate how important RIP is compared with other pathways in ErbB-4 signal transduction, and to see whether RIP is required for signaling through other tyrosine kinase receptors. Different members of this receptor family exhibit substantial overlap in their abilities to activate various SH2-domain signaling molecules. RIP offers one way to induce specific signals from different receptors (see the figure). Finally, it will be imperative to identify the nuclear partners of RIP-derived receptor fragments and their target genes.

References

1. C.-Y. Ni, M. P. Murphy, T. E. Golde, G. Carpenter, *Science* **294**, 2179 (2001).
2. M. S. Brown, J. Ye, R. B. Rawson, J. L. Goldstein, *Cell* **100**, 391 (2000).
3. H. Steiner, C. Haass, *Nature Rev. Mol. Cell Biol.* **1**, 217 (2000).
4. F. Oswald *et al.*, *Mol. Cell. Biol.* **21**, 7761 (2001).
5. P. A. Edwards, J. Ericsson, *Annu. Rev. Biochem.* **68**, 157 (1999).
6. X. Cao, T. C. Südhof, *Science* **293**, 115 (2001).
7. W. T. Kimberly, J. B. Zheng, S. Y. Guenette, D. J. Selkoe, *J. Biol. Chem.* **276**, 40288 (2001).
8. S.-Y. Lin *et al.*, *Nature Cell Biol.* **3**, 802 (2001).

PERSPECTIVES: MOLECULAR BIOLOGY

Methylation Talk Between Histones and DNA

Adrian Bird

Biological phenomena are complex, but biologists, being human, crave simplicity. Hence the frisson of excitement, mixed with relief, with the union of two hitherto separate domains of study—in this case, the methylation of DNA and the methylation of histone proteins. DNA methylation is a mark on genomic DNA made by addition of methyl groups to cytosine bases, whereas histone methylation marks proteins that coat the DNA by addition of methyl groups to certain lysine residues. In their recent *Nature* paper, Tamaru and Selker (*1*) report that DNA methylation and histone methylation share a common pathway in the filamentous fungus *Neurospora crassa*. Their discovery sets the stage for an experimental attack on one of the abiding mysteries of the genome: How do patterns of DNA methylation originate?

The problems inherent in managing large eukaryotic genomes can be eased by marking regions of DNA. Such marked DNA becomes structurally adapted so that it can perform certain activities. The most direct mark is one applied to the DNA itself, but this addition presumably must avoid adverse effects on the genome's stability and coding properties. The only marking system that is widespread among eukaryotes is methylation of DNA cytosine rings at position 5 to give the modified base, 5-methylcytosine (m^5C). More elaborate marking can be achieved by coating the genome with immobile proteins that are specifically designed to carry covalent messages. Core histones—the proteins assembled into the beadlike nucleosomes around which the DNA is wrapped—can be thought of as information modules that acquire coded information based on addition or removal of chemical groups. Their amino-terminal tails protrude from the nucleosome beads and can be modified through the attachment (or removal) of acetyl, phosphate, or methyl groups (*2*).

The resulting "histone code" affects the accessibility of the packaged DNA to transcriptional activator proteins, and hence the ability to switch on gene expression. For example, the lysine residue K9 in histone H3—the ninth amino acid from the

The author is at the Wellcome Trust Centre for Cell Biology, Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, UK. E-mail: a.bird@ed.ac.uk