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Meeting Report

Eleventh Annual Sapporo Cancer Seminar

Connecting the Dots: Assembling the Pathways That Control Cell Division

The challenges that face cancer research continue to be frighteningly difficult. As more knowledge of the fundamental changes that promote cancer has come to light, the problem of how tumors develop has become clearer. Yet this new resolution not only tells us new information that advances our understanding but also often tells us how little we actually know. At each stage, it would have been difficult to imagine how imprecise our view of the carcinogenic process was: not one oncogene, but many; not just positive regulation, but negative as well; multiple changes to promote each tumor; no required order for the multiple changes; different changes leading to apparently indistinguishable tumors. This field has moved from one more difficult realization to another. Yet standing in these difficult realizations are the most astonishing discoveries. The patterns laid down in determining the causes of human cancer have become the paradigm for the molecular signaling. The signaling processes that tell a cell when it is appropriate to divide are analogous to the signals that determine most, or perhaps all, molecular switches. As such, the difficult tasks of assembling these pathways hold enormous potential for all fields of biology.

All meetings on the basic causes of cancer ultimately rest on this goal, assemble the normal signaling pathways, and hopefully uncover the pathology of the cancerous ones. Obviously, the decision of a cell to divide depends on its ability to interpret extracellular signals. These signals are transduced by poorly understood pathways and allow the cell either to divide or not. The signals are both positive and negative, yet little is known of whether the decision rests on a balance of these signals or whether there is a master switch that determines the priorities of the multiple signals. One of the most pressing tasks facing oncologists is to assemble these pathways. Uncovering the plot of this play has been slow. Identify the characters, try to decipher their lines, learn their motivations and their partners, piece together small scenes, and perhaps eventually enjoy the whole show. This report covers one small assembly of would-be playwrights, experimenting with their small scenes. The subject of the 1991 Sapporo Cancer Seminar was "Molecules in Carcinogenic Processes."

From Pathways to Genes

The problem of establishing pathways of molecular communication is generally attacked by two general approaches, through developmental genetics or through building the pathway from the biochemistry of each protein. Perhaps one of the most exciting developments of the last several years is the realization that the same types of proteins or at least protein domains can be identified by these diverse approaches. Therefore, one expects that these signaling pathways will have universal tenets.

Understanding developmental pathways traditionally starts with a genetic screen identifying mutations that perturb the outcome of a developmental pathway. When mutant genes are cloned, they often begin to unravel into a pathway of interest. In a number of systems this work has uncovered a large number of genes which have all of the hallmarks of oncogene function: extracellular ligands that initiate a signal; receptors; kinases; intracellular secondary messenger systems; regulated translocation of key transcription factors; and transcription factors themselves.

One remarkable developmental system that promises to continue this alliance is the formation of the vulva in the worm, *C. elegans*. Here the theme is continued; characters or at least protein motifs that we know from the oncogene world are key elements in controlling this complex developmental pathway. At the Sapporo meeting, both Horvitz (MIT, Cambridge, MA) and Oshima (Kyusyu University, Fukuoka, Japan) discussed the identification of genes that play important roles in the development of the vulva in *C. elegans*. The development of the vulva depends on the appropriate cells interpreting several sets of signals. One signal comes from the binding of a soluble growth factor to its receptor on cells that will form the vulva. This soluble factor looks similar to the mammalian EGF,[1] and its presence sets up a diffusible gradient. Not surprisingly, the receptor for the EGF-like factor resembles an EGF receptor. The signal arising from the lin3 (EGF-like) binding to the let23 receptor (EGF receptor-like) is transmitted to the products of the *C. elegans sem5* and *let341* genes. The function of *sem5* is not known, but it contains both SH2 and SH3 domains.

The next step in the pathway involves the let60 protein, a *ras* homologue. Activating mutations of let60 that drive this pathway of vulva formation in the absence of the original lin3 signal are found primarily at codon 13 mutations, all changing the wild type glycine residue to the mutant glutamic acid. These same point mutations are known to activate the mammalian *ras* gene for transformation. Other mutations of let60 that inactivate the vulval development are also known. Some of these occur at sites that have no apparent analogue in mammalian *ras*, but codon 37 mutations give a recessive lethal phenotype. The codon 37 mutations hit what is thought to be the effector domain of mammalian *ras* and thus would make the let60 protein unable to pass on its signal to downstream targets. Activating mutations of let60 lead to multiple vulva formation, while inactivating mutations lead to vulvaless animals. Thus, mutations in a well characterized developmental pathway of *C. elegans* draw close parallels with the loss of control in cell division associated with human cancer. This system promises to allow the use of powerful genetic tests to decipher the pathway, a pathway which will hopefully have direct applications on understanding of the *ras* pathway in mammalian cell.

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[1] The abbreviations used are: EGF, epidermal growth factor; PI kinase, phosphoinositol kinase; PLC-γ, phospholipase C; MCC, mutated-in-colon carcinoma; APC, adenomatous polyposis coli; EGR, early growth response.

ras, *ras*, or *ras*

ras mutations that are associated with human cancer continue to provide important clues both for understanding transformation and for key steps in diagnosis. This idea was prominently displayed in Sapporo. Hirai (University of Tokyo, Tokyo, Japan) found that N-*ras* mutations were common in myelodysplastic syndrome. Peruchio (California Institute of Biological Research, La Jolla, CA) has now surveyed large numbers of pancreatic, colon, and lung tumors for mutations in *ras* that affect codons 12 or 13, two known hot spots of mutation for *ras*. His results continue to expand the instances where *ras* mutations are found frequently in some tumors but absent in others. Where they do develop, *ras* mutations can be seen more prevalently at certain stages of differentiation. Perhaps the most striking examples of these epidemiological conundrums are seen as Asp13 mutations that are prevalent in colon carcinoma but have never been seen in pancreatic tumors. Conversely, 75% of all pancreatic tumors that show K-*ras* position 12 mutations. The appearance of these pancreatic mutations also appears to correlate broadly, although not exclusively, with the state of differentiation of the tumor, with less differentiated tumors showing a high percentage of *ras* mutations.

Not only do *ras* mutations continue to be important diagnostic markers but they are also the testing grounds for new diagnostic assays. *ras* mutations provided the background for Sekiya (National Cancer Center Research Institute, Tokyo, Japan) in the development of the now widely used single-strand conformation polymorphisms. This technique measures different migration rates caused by single base changes in target DNAs. Using this technique, Sekiya was able to analyze rapidly fresh tumor specimens from 129 lung carcinomas for *ras* mutations. As mentioned below, the single-strand conformation polymorphism technique is now widely used for mutational studies.

When *ras* genes are introduced into mice as transgenes, they also become excellent targets for mutagenic events. Katsuki (Tokai University, Kanagawa, Japan) found that approximately 50% of the transgenic mice that carry the wild type *ras* gene under its own promoter develop tumors. This number can be raised by treating mice with mutagens such as 7,12-dimethylbenz(*a*)anthracene or *N*-nitroso-*N*-methylurea. Surprisingly, the mutations characteristically occur in the transgene and not in the endogenous gene. The resulting papillomas often have the codon 12 Gly to Val activating mutation.

Similar tumors can be found when the endogenous *ras* gene is mutated in standard skin carcinogenic studies using mutagens (Balmain, Beatson Institute for Cancer Research, Glasgow, Scotland). The timing of development of these mutations now gives us a better clue as to their impact. *ras* mutations in these systems are known to be initiating events in the carcinogenic process. However, later in the generation of the tumors, a second *ras*-related step occurs. The mutant allele is often amplified or the wild type allele is lost. These changes most often occur in the least differentiated tumors, a situation similar to what is seen in human pancreatic tumors (see above).

How does the mammalian *ras* pathway work? Much of our knowledge of this pathway stems from the biochemistry of the ras protein. As one example of the small guanine nucleotide-binding proteins, ras cycles through two major states during its signaling. In the GTP-bound state the protein is an active signaler. The GDP-bound state is inactive. Mutations that activate ras for transformation characteristically lock ras in the GTP-bound state and consequently hold the signal on. Advances in our knowledge of the biochemistry of ras have come from a number of sources. Three successful approaches were discussed at the Sapporo meeting.

In one, revertants of ras-transformed cell lines are studied. Both changes that are concomitant with the reversion of the cells and genes that will cause the reversions become important areas of study. Kusumaki (Hokkaido University, Sapporo, Japan) has noted that revertants of ras-transformed cells often have a mutated form of gelsolin, a M_r 92,000 protein the expression of which varies with the growth state of cells. Noda (Cancer Institute Japanese Foundation for Cancer Research, Tokyo, Japan) and his colleagues have isolated a gene that will rescue cells transformed by ras. This gene, called K-*rev*, is a relative of *ras*, and its ability to revert the transformed phenotype of the ras-transformed cells relies on its ability to act like *ras*. This is demonstrated most convincingly by chimeric studies. *ras* itself can be induced to rescue ras-transformed cells when a chimera that contains only a few amino acids from K-*rev* is introduced in an otherwise wild type ras protein. The key amino acids appear to be in a region immediately upstream of the so-called effector loop, a region that ras uses to interact with other proteins, including the GTPase-activating protein GAP and *NF1*, a recently identified gene that is causally linked to the development of neurofibrosarcomas. Both these proteins are active stimulators of ras GTPase activity, and both appear to interact with ras in the effector loop, the region of ras that is needed for effector function. However, these proteins appear to link ras function to very different events. McCormick (Cetus Corp., Emeryville, CA; now Chiron Corp.) reported that in addition to interacting with ras, GAP also interacts with tyrosine kinase receptor kinases. NF1 on the other hand appears to be more active as an GTPase-activating protein than GAP, but it is not clear to which signals the NF1 protein is responding. The NF1 protein activity does appear to be quite sensitive to lipid levels *in vitro*; thus it is tempting to suggest that these proteins may link ras activity to lipid-second messenger signaling.

The relation between ras and GAP can also be seen in genetic studies in yeast. M. Yamamoto (University of Tokyo, Tokyo, Japan) in an effort to identify proteins that will interact with ras, analyzed genes that could rescue ras mutation in *Saccharomyces pombe*. One of the genes that was isolated in this screen was a fission yeast GAP. This protein was more similar structurally to the mammalian NF1 than to GAP; thus it is not clear which mammalian gene is the closest homology. Interestingly, however, genetic studies indicated that the fission yeast GAP functions upstream of the ras action, suggesting that it might be involved in control of ras, rather than in its downstream signaling.

SH2 Domains, a Localization Domain or a Secret Handshake?

SH2 domains were originally defined as one of the regions of homology with src. They have been identified in an increasingly large number of proteins, some with seemingly little in common. At this meeting, the appearance of SH2 domains were discussed in the *C. elegans sem5* gene (Horvitz), GAP (McCormick), the M_r 85,000 regulatory subunit of phosphoinositol kinase (PI kinase; Oshu, Ludwig Institute for Cancer Research, London, England) and PLC-γ (Takenawa, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan). What are the roles of

this domain? First, SH2 domains appear to act as contact sites for protein interaction. However, SH2 interactions have have a second level of recognition. Binding to its target sequence requires a phosphorylated tyrosine residue in addition to the correct amino acid context. Thus, the SH2 domains allow targeting of one protein to another, but only after the action of a tyrosine kinase-mediated signal.

In three of the cases listed above, GAP, PI kinase, and PLC-γ, the proteins use the SH2 domains to translocate and bind to the receptor tyrosine kinases, such as the platelet-derived growth factor receptor. Platelet-derived growth factor receptor binding stimulates autophosphorylation of the receptor molecules on tyrosine to provide the correct target for SH2 binding, allowing binding of these three proteins. How the signal is transmitted following this translocation is less clear. An obvious target for GAP is the activation of the ras GTPase activity. Activated PLC-γ will hydrolyze phosphoinositol 2-phosphate and overexpression of a region of PLC-γ that contains the SH2 domain will cause stimulation of the hydrolysis levels (Takenawa). PI kinase phosphorylates phosphoinositol phosphate at a novel position on the inositol ring, creating a potential new second messenger. However, any effects of this messenger are still unknown. The demonstration of the SH2 domain in the regulatory subunit of PI kinase was made possible by the recent cloning of this gene by Oshu and colleagues. Cloning of the catalytic subunit is now under heavy attack by a number of laboratories.

src Family Kinases

Signaling from the cytoplasmic tyrosine kinases still remains a mystery. However, the upstream regulation of these kinases is beginning to be sorted out. Two types of regulatory events are now known. T. Yamamoto (University of Tokyo) described recent results of one of these signaling mechanisms. The initiation of signals in lymphocytes has been a major area of study for many years. Yamamoto described the interaction of members of the src family of tyrosine kinases with well characterized cell surface signaling molecules of lymphocytes. In T-cells the fyn kinase binds to the T-cell receptor/CD3 complex following T-cell activation. In B-cells the lyn kinase binds to membrane-bound IgM and IgD molecules. Here, stimulating B-cells by adding antibodies specific for the IgM μ chains increases tyrosine phosphorylation of many cellular proteins including the lyn kinase. Thus, it appears that the tyrosine kinases fyn and lyn are likely to be important molecules that deliver signals following activation of T- and B-lymphocytes.

A second upstream control mechanism of the src family kinases is an internal phosphorylation of a key tyrosine residue. For src, tyrosine 527 is phosphorylated to hold the intrinsic kinase activity of src inactive. Workers have searched diligently for the kinase that phosphorylates this residue, and Okada (Osaka University, Osaka, Japan) reported the isolation of a cellular kinase that will specifically phosphorylate this site. The kinase was isolated from brain and now its complementary DNA has been cloned. Surprisingly, this new kinase resembles the src family kinases themselves, although it does not have a site equivalent to the 527 negative regulatory site of src. *In vitro* this kinase, known as CSK, is able to phosphorylate analogous regulatory sites on other src family kinases, including fyn, yes, lyn, and lck. However, the CSK kinase is specific for these sites, inasmuch as it will not phosphorylate other tyrosine phosphorylation sites.

The importance of tyrosine phosphorylation to transformation is well established. Nishimura (National Cancer Center Research Institute) reported preliminary findings using an antityrosine drug, azatyrosine, on the effects of reversing tyrosine phosphorylation. Azatyrosine treatment of many transformed NIH3T3 cells will revert these cells to normal phenotypes. They have a flat morphology, no longer grow in soft agar, and are nontumorigenic. Perhaps most surprisingly, these phenotypes appear to be stable; azatyrosine treatment does not need to be continuously administered. To date, Nishimura has shown that K-*ras*-, N-*ras*-, c-*raf*-, and *erbB*-2-transformed cells can be reverted. *hst*-, *src*-, or *ret*-transformed cells cannot. In transgenic H-*ras* mice, he reported that NMU-induced papillomas could be inhibited in 100% of the cases with cotreatment with azatyrosine. DMBA-induced papillomas were reduced to 50% of the treated mice. Recent attempts to isolate a more potent compound have discovered one, SF2698, which is approximately 10 times more active than azatyrosine. No animal studies have yet been done with SF2698.

Fighting Back the Kinases

While protein phosphorylation is a well studied phenomenon known to be actively involved in signal transduction, less is known about the enzymology of phosphate removal. Two major groups of phosphatases are known. These are the tyrosine phosphatases and the serine/threonine phosphatases. Unlike the tyrosine and serine/threonine kinases, which are structurally related, these two groups of phosphatases appear to be unrelated.

While both types of phosphatases have been known for some time, studies of their molecular biology are still in their early stages. Nagao (National Cancer Center Research Institute) reported the cloning of the catalytic subunits of 4 type I and 2 type II serine/threonine phosphatases. Early functional studies of the effects of blocking phosphatase activity have shown that type II activity is important in certain types of NIH3T3-transformed cells. Treating transformed cells with okadaic acid at low concentrations, conditions that specifically inhibit type II phosphatases, led to reversion of several phenotypes, including morphology. Flattening of cells transformed by *ret*, *raf*, or polyoma virus was seen following okadaic acid treatment.

The field of tyrosine dephosphorylation is an even more recent hotbed. The last year has seen the identification and cloning of a large number of tyrosine phosphatases from many different sources. Proof that these enzymes serve a purpose in signal transduction is still lacking, but many circumstantial facts suggest that the proof will be seen shortly. Perhaps the most suggestive evidence comes just from the structure of the new tyrosine phosphatase clones. Several of these are transmembrane molecules with large extracellular domains that resemble well known signaling molecules from other sources (Tonks, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). Functionally it is clear that deregulated forms of the tyrosine kinases have dramatic effects on cell activities. Tonks reported that a deregulated form of a T-cell tyrosine phosphatase inhibited mitosis and cytokinesis, producing cells that often contained multiple nuclei in many different stages of the cell cycle. What remains to be determined is how regulation of tyrosine dephosphorylation occurs and what processes depend on the action of such potent enzymes.

Telling Cells When to Stop

Our knowledge of how cells learn when not to divide is still quite limited. Cells in many circumstances clearly need to learn when to cease growth, but these signaling pathways are still sketchy. While the number of proteins that have been identified in negative growth regulation has increased in recent years and months, the process of cloning these genes has been slow and very difficult. Each new example has come with monumental effort, and the results therefore seem somehow more special because of the technical achievement.

One example of a gene in search of this recognition is the MCC gene, discussed by Miyoshi (Cancer Institute Japanese Foundation of Cancer Research). Inheritance of a mutated gene at 5q21 predisposes patients to APC. Two genes have now been cloned from this area, one by Miyoshi and colleagues. The MCC gene encodes a protein of 829 amino acids. As expected from its name, certain patients with colon carcinomas show sequence differences in this gene, but the numbers are low (only 2 potential mutations in 100 tumors). More telling is that no mutations in patients with APC seem to carry mutations in MCC. While the possibility of contributing to the development of a small percentage of colon tumors is still tenable, it does not appear that this gene will account for the APC phenotype.

The identification of other tumor suppressor gene candidates continues to be a major and important goal. Yokota discussed his group's progress in the cloning of a tumor suppressor gene on chromosome 3p, a locus that has been linked to many different tumor types.

Tumor Suppressor Gene Function

The last several years has seen the study of tumor suppressor genes move forward in two dramatic ways. As discussed above, several new tumor suppressor genes have been cloned, and the nuclear tumor suppressor genes have moved into the realm of transcriptional control.

The tumor suppressor gene products that are localized to the nucleus include the retinoblastoma protein (pRB), *p53*, and Wilms' tumor (WT1). All three of these proteins are now implicated in transcriptional control. Their study has been highlighted by an attempt to learn more of their biochemical functions. This has been most direct in the case of the WT1 gene. At Sapporo, Rauscher (Wistar Institute, Philadelphia, PA) and Haber (Massachusetts General Hospital Cancer Center, Charlestown, MA) both discussed work that brought the function of the WT1 protein into a clearer light. Wilms' tumors are a type of childhood kidney tumor that are characterized by loss of one or more different genetic loci. Only one of these loci has been cloned, and it is found on chromosome 11p13 (Haber). The RNA from this gene is expressed primarily in the developing kidney and is differentially spliced to yield 4 mRNAs. The molecular weights of the WT1 proteins are between 50,000 and 54,000 and have sequence homology with a putative transcription factor known as EGR. This homology is limited to a zinc finger domain. EGR was originally identified as one of the immediate early genes the transcription of which is activated after treating growth-arrested cells with serum. EGR is thought to activate transcription; consequently, its homology with WT1 provides an interesting contrast for study. Rauscher reported the activities of WT1/EGR chimeras. As expected all the chimeras could bind to the same DNA sequence, inasmuch as their homology appears to define a conserved DNA binding domain. Combining either DNA binding domain with the remaining portions of WT1 or EGR changes the function of these proteins. EGR chimeras with either DNA binding domain are potent activators, while the analogous WT1 chimeras are potent repressors. These results suggest that these proteins, or ones with similar structures, are antagonists. The results also imply that the targets of action of these proteins are a key next step in these studies.

Another strong indication of this was presented by Haber. Naturally occurring mutations in Wilms' tumor patients are beginning to provide the needed reagents to look for the key regions that trigger signaling events. Most patients have one mutant allele with one deleted allele at the WT1 locus. One patient displayed an interesting difference. In this case one allele was wild type and one was mutant. In order to preserve the predicted loss of function in this gene product, it is necessary to suggest that the mutation is a dominant negative allele. If true this mutation would interact with a key element in WT1 signaling and block further transmission. This mutant protein fails to bind to DNA and thus must interact with a signal transducer other than DNA. Perhaps this will provide the needed reagent to identify other important molecules in the WT1 pathway.

This system is reminiscent of another well studied tumor suppressor gene, *p53*. Here, there are good examples of growth inhibition following the introduction of wild type *p53* into cells. Likewise, in transfection experiments it appears that one can introduce *p53* mutations that will counter the function of wild type proteins and remove the growth-inhibitory effects of wild type protein. In recent work, Oren (Weizmann Institute of Science, Rehovot, Israel) has demonstrated a surprising outcome when *p53* is introduced into certain cell types. When Oren introduced wild type *p53* into mouse M-1 myeloid cells to check for *in vitro* differentiation, he finds that the cells enter apoptosis or programmed cell death. Oren speculated that this may provide a physiological role to the ability of *p53* to inhibit cell growth, to initiate a mechanism in which stem cell populations could be controlled by programmed cell death.

Tumor Suppressor Gene Diagnosis

Scoring for changes in known tumor suppressor genes continues to be a important area in the quest for correlations between tumor development and detecting causal mutations. The most frequently analyzed gene in these types of studies is clearly *p53*. Recent work from Balmain's and Sekiya's laboratories suggests that *p53* mutations at least in several systems appear late in the development of certain tumors. Balmain, working in the mutagen-induced skin papilloma system, and Sekiya, studying human hepatocellular carcinomas, both found no incidence of *p53* mutations in early stages of the tumor development. This suggests that *p53* mutations must not have a selective advantage in early stages. This appears not to be the case for all tumors, however. The opposite conclusion was reported by Yokota (National Cancer Center Research Institute) looking at small cell lung carcinoma, where *p53* mutations could occur at early or late stages.

The importance of mutations in certain tumor suppressor genes was emphasized by the high frequency of finding these mutations in certain tumors. Yokota reported that nearly 100% of the cases of small cell lung carcinoma showed mutations at 3p, 13q, and 17p, the sites of an as yet uncloned tumor suppressor gene, the retinoblastoma gene, and *p53*, respectively.

p53 mutations were detected in many tumor types, but Ludeke (Massachusetts Eye and Ear Infirmary, Boston, MA) reported an interesting parallel between the types of mutations that are found and their tissue of origin. In most tumors they had studied, the *p53* gene carried point mutations, but in osteosarcomas they saw a large number of gross rearrangements. The most complete panel of different mutation types were analyzed by Ludeke in the retinoblastoma gene. All somatic mutations of the *RB1* gene lead to inactivation of the gene, producing nonsense terminations, frameshift mutations, or substitutions in splice site consensus sequences. However, for germinal mutations, 32 lead to new stop codons or frameshifts, 7 were within canonical splices sites, and 4 were missense mutations.

The clear need to disable tumor suppressor gene products was demonstrated in a novel manner by Munger (National Cancer Institute, Bethesda, MD). Munger and his colleagues have been studying cervical carcinomas that arise either sporadically or following earlier infections with certain human papilloma viruses. Human papilloma virus encodes two potent oncoproteins that bind specifically to *p53* and pRB. It is thought that these interactions inactivate these proteins and thus mimic the loss of their genes seen in many human tumors. Munger now has analyzed a panel of cervical carcinoma cell lines and finds a remarkable correlation. Every cell line either carries a mutation in both *p53* and pRB or expresses the viral proteins. This is the best evidence yet that loss of *p53* and pRB function is important for the development of cervical carcinomas. It also argues strongly that the interactions with these viral proteins are likely to be true inactivating events.

The retinoblastoma protein itself appears to be under tight temporal control in growing cells. Although the actual function of pRB is still not clear, several workers have now shown that a major upstream regulator of its function are cell cycle-controlling kinases such as cdc2. Taya (National Cancer Center Research Institute) and Akiyama (Osaka University, Osaka, Japan) both reported that the cdc2 kinase could phosphorylate pRB *in vitro* in a manner that was analogous to the sites phosphorylated *in vivo*. Taya also was able to show that pRB could be isolated in a physical complex with cdc2 itself as well as with another protein that resembled cdc2, at least by immunochemical properties.

Cell Cycle Meets the Oncogene

One of the major questions in cell cycle research is, "Where will the regulatory events of the cell cycle interface with genes that signal cell proliferation?" The control of the retinoblastoma protein appears to be one spot of interface, but others are also known. Pines (Salk Institute, San Diego, CA) reported on the cloning and characterization of the human cyclins. Cyclins are regulatory subunits that interact with kinases such as cdc2 to yield cell cycle-regulating enzymes. How cyclins control the kinase activity of cdc2 is largely unknown. One new clue to this story was provided by Pines when he showed that the localization of the various cyclin/cdc2 complexes varied during the cell cycle. Most dramatically, he showed that the cyclin B/cdc2 complex remains in the cytoplasm until the beginning of mitosis, when it is translocated to the nucleus. This places it in the correct subcellular locale with most of its key mitotic substrates.

Perhaps the best understood of the cell cycle connections with oncogenes comes from the analysis of the mos kinase. This kinase was originally identified as the transforming agent of the mouse Moloney retrovirus. Ikawa and colleagues (Tokyo Medical and Dental University, Tokyo, Japan) have shown that this same kinase is a key component of oocyte maturation. The mos kinase is a major component of cytostatic factor, until recently a poorly characterized factor that inhibited further cell cycle progression in unfertilized eggs. Active mos kinase is a key component of cytostatic factor.

Closing with the Opening

The two first talks of this meeting were overviews given by Sugimura (National Cancer Center) and Vogt (University of Southern California, Los Angeles, CA). Although these talks set the stage for our discussions at Sapporo, for me they seem like the best closing, not just from what they taught us but also from what questions they brought to mind. Sugimura, by discussing the multiple steps needed for carcinogenesis, reminds us of the strong natural selection that leads to tumor formation. In particular, for tumor cells to succeed, they represent a culmulation of many mutations, each releaving a barrier to growth. Vogt discussed the selection pressures faced by retroviruses and the barriers that they overcome by transducing oncogenes. His main point was that nuclear oncogenes function by deregulating key transcriptional events. For me, his talk stimulated a question with sinister overtones. How narrow is our knowledge of oncogenes and tumor suppressors? Have retroviruses, DNA transfections, and tumor suppressor gene hunts uncovered the majority of genes that contribute to cancer? Or can we expect to be surprised by the next step of our learning curve on carcinogenesis? How many ways can loss of regulation occur and have we encountered the majority of them? As an audience watching the discovery of this grand play, we have much to learn.

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