Meeting Report

Regulation of nuclear functions – nucleocytoplasmic transport in context

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A recent meeting of cell biologists in Cleveland, Ohio (April 22/23, 2004) highlighted the value of juxtaposing discussions of the functional organization of the nuclear periphery with the mechanisms by which the nucleus receives signals, both from the cell surface and from defined sites in the cytoplasm. The event was jointly organized by the Cell Biology Program of Case Western Reserve University School of Medicine and the Cell Biology Department and Lerner Research Institute of the Cleveland Clinic Foundation.

In introductory remarks, Alan Tartakoff (Case Western Reserve University) highlighted the breadth of importance of dynamic nucleocytoplasmic communication. He described contemporary molecular understanding of "signaling" to the nucleus as one of the recent "conquests" of cell biology, which characteristically insists on mechanistic questions which involve topologic or topographic issues. He also called attention to the realization that many examples of nuclear import and export do not directly involve the "classic" transport pathways which are driven by the Ran GTPase and members of the family of importins/exportins (also known as "karyopherins") (Görlich and Kutay, 1999; Xu and Massague, 2004). He further emphasized that net relocalization of proteins from the nucleus to the cytoplasm (and vice versa) can – in many cases – result from adjustment of the rate constants of ongoing shuttling, rather that being due to "de novo" unidirectional transport (Fig. 1) (Tartakoff et al., 2000). As a prelude for subsequent lectures, he then enumerated additional circumstances which can cause individual transport cargoes to distribute asymmetrically across the nuclear envelope: unmasking of transport signals and protein-protein interaction domains (due to covalent changes or displacement of inhibitory peptide domains), tethering of cargo proteins to immobile structures (either covalently or non-covalently), etc.

The nuclear periphery

Distinctive membrane domains
Multiple proteins of the inner nuclear membrane (INM) of higher eukaryotes interact with lamins, which are concentrated
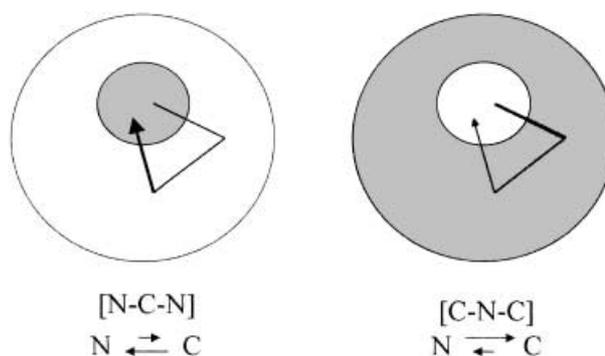

Fig. 1. Shuttling options. The cell at the left illustrates the distribution of a shuttling protein whose rate of import exceeds its rate of export (e.g. hnRNP A1). The cell at the right illustrates the distribution of a shuttling protein whose rate of export exceeds its rate of import (e.g. PKI or other proteins which shift to the nucleus upon addition of leptomycin B, the inhibitor of the exportin, Crm1). Perturbation of either the import or export rate constants can readily cause massive redistribution of the proteins without interrupting their shuttling.

at the periphery of the nucleoplasm, and appear to restrict the diffusional loss of INM proteins to the ER. Judging from recent studies of yeast, the organization of peripheral proteins may also be central to censoring the export of pre-mRNAs (Galy et al., 2004).

After reviewing the evidence of tethering of individual INM proteins to lamins, Brian Burke (University of Florida) presented evidence that membrane proteins can also localize specifically to the outer nuclear membrane (ONM), despite the widely-held belief that the protein composition of the ONM is identical to that of the ER (Mounkes et al., 2003; Georgatos, 2001; Worman and Corvalin, 2004; Salina et al., 2001; Moir et al., 2000). The best-characterized ONM candidates to date are the nesprins (Fig. 2), of which there are many forms which result from alternative splicing, ranging up to 800 kDa ("giant"). Sequence comparisons demonstrate their relation to Anc1 (*C. elegans*) and Klarsicht (*Drosophila*) (Starr and Han, 2003; Lee et al., 2002; Patterson et al., 2004; Myat and Andrew, 2002; Padmakumar et al., 2004; Muchir et al., 2003; Bengtsson and Wilson, 2004). These proteins have previously been implicated in interactions with the actin cytoskeleton and in positioning of the nucleus within the cell. Evidence of their

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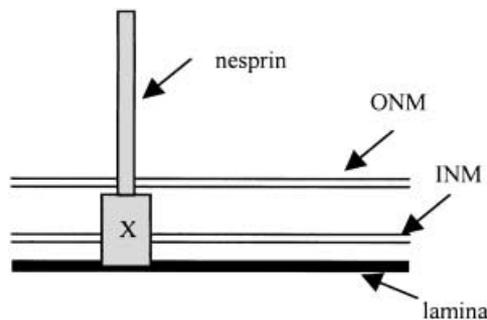
Fig. 2. Diagram of INM/ONM interactions. The nesprins are trans-membrane proteins which interact with the actin cytoskeleton, as well as with proteins of the inner nuclear membrane (INM) and lamins. X is a hypothetical transmembrane protein which can link the nesprin to lamins and other proteins in the nucleoplasm. The diagram suggests mechanical linkage spanning from the cytoplasm to the nucleoplasm.

linkage to constituents within the lumen of the nuclear envelope, to INM proteins and to lamins comes from biochemical studies and transfection experiments in which overexpression of their cisternal "KASH" domains causes both nesprin "giant" as well as proteins of the INM (LAP2, emerin, etc.) to relocate to the ER. The existence of interacting proteins which span the cisternal space of the nuclear envelope may make it possible for cytoplasmic signals to affect components at the periphery of the nucleoplasm. Trans-cisternal interactions may also contribute to the maintenance of relative constancy of width of the lumen of the ER.

The importance of lamin A for integrity of the nuclear periphery and normal physiology was demonstrated by studying lamin A knock-out mice. Although viable, these animals grow only slowly and exhibit severe muscular atrophy, possibly comparable to that caused by dominant mutations in human lamin A or interacting proteins (e.g. emerin). In the absence of lamin A, the nucleus of murine cells is often misshapen, the lumen of the nuclear envelope is focally dilated, and several proteins which normally reside at the nuclear periphery relocate to the ER.

Much more extensive distortion of nuclear shape, dilation of the nuclear envelope, displacement of proteins which normally are found in the INM, as well as muscular dystrophy result from knocking in mutant forms of lamin A which are characteristic of human laminopathies. As in the human laminopathies, the cell type-specificity of these changes is puzzling.

Alan Tartakoff also presented evidence that the shape of the yeast nucleus can be dramatically modified by modulating the expression of proteins which normally localize to the periphery. These observations may provide the basis for a genetic approach to analysis of the integrity and shape of the nucleus.

Importin interactions with cargo and nucleoporins

Many proteins containing nuclear localization signals (NLSs) are transported into the nucleus by the nuclear import receptor importin β. Janna Bednenko (The Scripps Research Institute) summarized structural information on the interaction of the three types of NLS (the classical IBB domain of importin α, PTHrP, and SREBP-2) with importin β. Each of these NLSs binds a distinct part in the inner surface of importin β, which is lined by the short B helices of HEAT repeats (Cingolani et al., 1999, 2002; Lee et al., 2003).

Since interaction with the Ran GTPase is essential for the function of import and export receptors, it is not surprising that all of them contain an N-terminal Ran-binding domain. It has been proposed that all the transport receptors have evolved from a common progenitor containing such a domain. The fact that an N-terminal portion of human importin β is functional for nuclear import of PTHrP provides experimental evidence for the evolutionary model (Cingolani et al., 2002).

Importin β carries its cargoes through the nuclear pore complex (NPC) using its sequential interactions with the FG (Phe-Gly) rich regions of nucleoporins. Structural studies have defined an FG repeat-binding region in the N-terminal portion of importin β (Bayliss et al., 2000). Using the structural alignment of the two halves of importin β and site-specific mutagenesis, Bednenko et al. identified a second nucleoporin-binding region in the C-terminal portion of importin β. Although the affinity of the C-terminal nucleoporin-binding site is much weaker than that of the N-terminal site, both of these sites appear to be important for importin β function. Bednenko et al. therefore have proposed a "monkey" model, in which the two nucleoporin-binding sites on importin β are used successively to promote its movement through the NPC. Thus, importin β does not lose contact with the NPC as it progresses from one FG repeat nucleoporin to another. It is possible that the presence of two nucleoporin-binding sites is a general feature of all the nuclear transport receptors (Bayliss et al., 2000; Bednenko et al., 2003a, b).

Insertion of nuclear pores into the nuclear envelope

Although all eukaryotic cells appear to have the possibility of increasing the number of NPCs during interphase, little is known about the mechanism by which individual nucleoporins (or individual NPCs) are inserted. By contrast, the conspicuous events of nuclear envelope breakdown and reformation which occur at the end of each cell cycle in higher eukaryotes have been studied extensively.

Douglass Forbes (University of California, San Diego) reviewed the structure of the nuclear pore, including evidence that nucleoporins Nup98 and Nup153 are critical for nuclear export. She provided an overview of her now-classic procedures which make it possible to reconstitute nuclei from extracts of *Xenopus* eggs when incubated with double-stranded DNA or chromatin. Both soluble factors and membrane vesicles are required for nuclear assembly. In addition, affinity depletion of individual soluble components prior to assembly makes it possible to assess the importance of specific pore proteins for assembly and function.

Recent studies by her group have identified a subcomplex of nucleoporins which is key for initiating NPC insertion (Harel et al., 2003b; Walther et al., 2003a). This "107 – 160" complex, identified and retrieved in the case of the Forbes lab through its affinity binding to insolubilized Nup98 or Nup153, was found to be a central determinant in nuclear pore assembly. Specifically, when nuclear reconstitution is performed in the absence of the 107 – 160 complex, the nuclei remain small, cannot import, and fail to incorporate other nucleoporins, including the membrane-spanning nucleoporins (gp210, POM121). High-resolution scanning EM demonstrates the nuclei to be membrane enclosed, but devoid of nuclear pores (Harel et al., 2003b; Walther et al., 2003a).

Considering the broader picture, correct nuclear pore assembly presumably requires an inner/outer nuclear mem-

brane fusion step which relies on interaction between proteins on the lumenal face of the inner membrane with ones on the lumenal face of the outer nuclear membrane, i.e., between INM and ONM proteins. Given the data presented by Forbes, such interactions likely precede the addition of most nucleoporins.

In a second thrust, Forbes presented results demonstrating that multiple steps in nuclear assembly are regulated by importinβ and Ran. It is now clear that these proteins have different functions during different parts of the cell cycle. During interphase, importin β and Ran function in nucleocytoplasmic transport. Early in mitosis, they control assembly of the spindle (Li and Zheng, 2004; Li et al., 2003; Zhang et al., 2002a, b; Askjaer et al., 2002). Focusing on nuclear assembly, the Forbes group has now found that excess importin β does not interfere with vesicle recruitment to the surface of chromatin, but blocks the vesicle-vesicle fusion required to form a double nuclear membrane. Ran counteracts this inhibition, promoting vesicle-vesicle fusion and resulting in nuclei which have abundant membrane invaginations into their interior.

In a fourth role, studies by both the Forbes (UCSD) and Mattaj (EMBL) groups, importin β negatively regulates NPC assembly/insertion (Harel et al., 2003a; Walther et al., 2003b). The take home lessons thus appear to be that importin β and Ran are global regulators of nuclear identity, in part owing their activity to the ability of chromatin-bound Ran GEF (RCC1) to concentrate Ran(GTP) at its surface. Forbes concludes that the cell uses a precise ratio of importin β to Ran to assure correct spatial and temporal assembly of the nuclear envelope and nuclear pores.

Complex transport itineraries of soluble cargoes

Among the most complex itineraries is that which is responsible for ribosome biogenesis. In this case, multiple ribosomal proteins are imported to the nucleolus, where they assemble with newly-synthesized rRNA while it undergoes covalent processing, and are subsequently exported as individual subunits. Export is apparently accompanied by concealment of all of the NLSs which mediate import of the individual proteins.

snRNP biogenesis
Greg Matera (Case Western Reserve University) described the complex subcellular itinerary of newly-synthesized splicing Sm snRNPs, which involves export of the RNA transcript to the cytoplasm, followed by assembly of the Sm-ring, hypermethylation of the 5′ cap, 3′ end trimming and reimport to the nucleus. After arrival in the nucleoplasm, newly-imported snRNPs target to Cajal bodies for additional modification steps before proceeding on to the sites where they function in pre-mRNA splicing in perichromatin fibrils. Recent work has shown that the cytoplasmic RNP maturation steps are linked to the survival of motor neurons (SMN) protein complex, which – for unknown reasons – is also found in the nucleus. SMN is mutated in SMA (spinal motor atrophy) and the mechanism by which it is imported into the nucleus is unknown. Using digitonin-permeabilized cells, he showed that SMN import is coupled to that of the snRNPs. Through the use of depletion and addback experiments he demonstrated that SMN binds directly to importin β via a domain previously implicated in Sm

protein binding. Several mutant forms of SMN which cause SMA are defective for import. These findings shed light on the mechanism of snRNP import, implying the existence of two independent pathways. Finally, he highlighted the major question in the SMA field – namely, how mutations in a protein involved in a process that is central to all cells can have such a tissue-specific phenotype. This concern is reminiscent of the cell-type specificity of laminopathies mentioned above.

Shuttling export escorts
Importin β can deliver cargoes to the nucleus which bind it directly or bind via adaptors (importin α, snurportin, etc.). Although less emphasis has been placed on adaptors which function in nuclear export, several adaptors are known which mediate the interactions between export cargoes and the exportin, Crm1, which exports proteins with "leucine-rich" export signals. Among these are the protein kinase A inhibitors (PKI) which include leucine-rich export signals and are small enough to diffuse into the nucleus (Wen et al., 1994) (Fig. 3).

Protein kinase inhibitor
Edward Greenfield (Case Western Reserve University) described the impact of parathyroid hormone (PTH) on osteoblasts, which is mediated by the PTH receptor, leads to increased cAMP concentration, stimulation of protein kinase A, and entry of PKA into the nucleus. Termination of signaling by PKA can be accounted for by its interactions with PKI-γ. Thus, reduction of PKI-γ using siRNA increases the nuclear titer of PKA. Nevertheless, the impact of PKI on PKA-dependent gene expression does not appear to be affected when nuclear export of PKI is blocked with leptomycin B (Chen et al., 2002).

p53 export
Regulated nuclear export of the tumor suppressor protein p53 governs its stability and ability to promote G1 arrest and apoptosis. However, the mechanisms that control p53 nuclear export have not been fully clarified. Carl Maki (University of Chicago) reviewed various models for p53 nuclear export that have been proposed and showed data that indicate MDM2-mediated ubiquitination of p53 promotes p53 nuclear export. Recent studies suggest that monoubiquitination leads to its nuclear export, while polyubiquitination promotes p53 degradation in the nucleus. Because p300 has been shown to promote
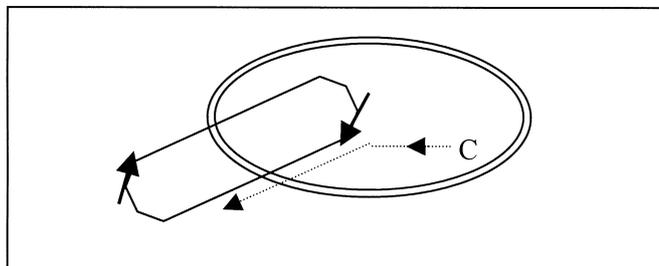

Fig. 3. Export escorts. In the cartoon of the nucleus (bounded by a double membrane), the oblong intersecting circuit toward the left designates the repeated nuclear entry and export of a shuttling protein (escort) which itself binds and is exported by an exportin. The export of a cargo (C) can depend on whether it binds to this "escort" and accompanies it to the cytoplasm.

p53 polyubiquitination, it was suggested that p300 binding regulates p53 nuclear export by controlling its ubiquitination. Consistent with this possibility, a p53-mutant lacking its p300-binding domain was highly susceptible to MDM2-mediated nuclear export, and was ubiquitinated to a lesser extent.

Once exported, p53 is thought to bind cytoplasmic anchor proteins such as PARC and the glucocorticoid receptor (if liganded). The latter interaction may result in each of the two proteins opposing the other's entry into the nucleus. Glucocorticoid antagonists may therefore be useful for promoting release and nuclear entry of p53, making it possible to sensitize cells, e.g. to radiation. Consistent with this possibility, the glucocorticoid receptor antagonist mifepristone (RU486) promoted nuclear entry of p53 in neuroblastoma cells and sensitized these cells to radiation-induced killing.

Mdm2 transport

Although the concentration of Mdm2 in the nucleus had been thought to reflect passive processes, it is now evident that growth factor-mediated PI3K/Akt signaling promotes localization of Mdm2 to the nucleus. Lindsey Mayo (Case Western Reserve University) indicated that Akt can phosphorylate Mdm2 near its bipartite NLS, as confirmed by in vitro and in vivo experiments using mass spectrometry and site-directed mutagenesis of the respective Akt phosphorylation sites (serine 166 and 186). Blocking Mdm2 nuclear entry (using dominant negative p85, a subunit of PI3K, kinase dead Akt, PTEN, Wortmannin, or LY294002) reengaged p53 to mediate cell cycle arrest, and to induce cell death in response to genotoxic stress. Nuclear Mdm2 can also return to the cytoplasm in response to growth factors, e.g. due to the impact of a mitogen-activated protein kinase pathway whose activation is delayed compared to Akt. Since inhibition of Mdm2 nuclear export stabilizes nuclear p53, constant growth factor stimulation, which is evident in tumors, can promote relocation of Mdm2 to the cytoplasm and thereby attenuate the ability of p53 to induce cell cycle arrest and apoptosis.

FOXO, apoptosis and longevity

Anne Brunet (Stanford University) focused on mechanisms which may account for the observation that caloric restriction increases life span in a wide variety of animal species. This observation has been linked to the insulin-like growth factor receptor (IGF1-R) because inhibition of this receptor in worms and mice leads to prolonged life. Brunet described the impact of IGF-1, which increases PI3K levels, stimulating PDK1, which in turn phosphorylates and activates the ser/thr kinases Akt (PKB) and SGK. Since IGF-1 activation of Akt causes it to enter the nucleus, Akt can phosphorylate a variety of factors which are sequestered there. Among these is the forkhead protein, FOXO3, whose phosphorylation by Akt and SGK subsequently causes it to leave the nucleus within minutes. Judging from experiments with phospho-amino acid-specific antibodies, its exit appears to be escorted by the 14 – 3-3 protein which binds to a phosphorylated residue near its N-terminus (Brunet et al., 2002).

To explore the consequences of nuclear localization of FOXO3 in the absence of IGF-1 signaling, Brunet expressed a fusion of FOXO3 with the ligand-binding domain of the estrogen receptor. The fusion protein can be shifted from the cytoplasm to the nucleus by addition of tamoxifen. Use of such protocols in conjunction with microarrays demonstrates that nuclear FOXO3 upregulates genes implicated in cell cycle arrest, DNA repair, detoxification of reactive oxygen species, as well as apoptosis.

Other circumstances which signal to FOXO3 include exposure to stress (e.g. heat shock), which causes FOXO3 to remain in the nucleus and undergo deacetylation by SIR2. Studies making use of SIR2 knock-out cells indicate that SIR2 enhances the ability of nuclear FOXO3 to promote stress resistance and prevents FOXO3 from inducing apoptosis. These and related observations show that the nucleocytoplasmic distribution of FOXO3 is pivotal for determining the balance between cellular resistance to stress and apoptosis, a balance that may be critical in regulating organismal longevity (Brunet et al., 2004; Tran et al., 2002).

Regulation of signaling from cytoplasmic sites

Multiple aspects of signaling from mitochondria to the nucleus have been explored in depth (e.g. (Kelly and Scarpulla, 2004)). These studies document the complex means by which the metabolic load on organelles can be transduced into signals which maintain organelle number and composition.

ER signaling to the nucleus

David Ron (New York University) began with a broad overview of the need for homeostatic signaling pathways which allow the ER, mitochondria and the cytosol to maintain appropriate titers of factors which aid the conformational maturation of "client proteins" – and provided evidence that such signaling does occur. He then described in detail the "Unfolded Protein Response" (UPR) of the ER which both attenuates protein synthesis and leads to induction of compensatory transcripts when there is imbalance between the titer of maturation factors and client proteins within its lumen.

In higher eukaryotes, three ER membrane proteins have been identified which are central to the UPR: PERK, IRE1 and ATF6. ATF6 (like SREB) undergoes intramembrane proteolysis (after arrival in the Golgi) to release a cytosolic moiety which then enters the nucleus and regulates transcription. Curiously, the higher eukaryotic UPR appears not to rely primarily on the homologue of yeast IRE1 (which is central to the unorthodox pre-mRNA splicing in the yeast UPR). Mammalian IRE1 signals via factors (XBP1) whose significance is only poorly understood, although genetic evidence suggests that XBP1 is required for elaboration of the equipment of the secretory path during plasma cell differentiation.

PERK the third component of the UPR, which was the focus of the talk dimerizes upon ER stress, resulting in its transphosphorylation, which allows it to act as an eIF2α kinase, thereby inhibiting the translation of most mRNAs. Nevertheless, at least one mRNA, that of the transcription factor ATF4, is upregulated by eIF2α phosphorylation. The encoded protein functions in the nucleus to activate gene expression in a branch of the UPR that is also accessed by other stresses that lead to eIF2α phosphorylation (referred to as the "integrated stress response"). Evidence of the importance of PERK is provided by studies of PERK knockout cells and mice. An approximate parallel with the roles of yeast GCN4 is evident in the functions of ATF4, a downstream nuclear effector of PERK signaling. PERK dimerization itself upregulates transcripts which are implicated in responses to oxidative stress and are

characteristic of amino acid and thiol sufficiency. A further target, GADD34, functions as an eIF2α phosphatase and thereby reinstates translation initiation, closing a negative feedback loop. Herpes virus exploits a related strategy to sustain protein synthesis in infected cells (Harding et al., 2000, 2001, 2003; Urano et al., 2000a, b).

Nucleocytoplasmic distribution of RNA-binding transcription factors

Paul DiCorleto (The Cleveland Clinic) reported on a novel mechanism by which the nucleocytoplasmic distribution of a transcription factor – and hence its activity – can be regulated. The Y-box transcription factor dbpB, a 50 kDa protein with DNA- and RNA-binding domains, is normally sequestered in the cytoplasm bound to mRNA. In endothelial cells, activation of the thrombin receptor, PAR-1, leads to the proteolytic cleavage of dbpB to generate a 30-kDa fragment. This truncated form no longer binds RNA. Since it contains a nuclear localization signal, it can translocate to the nucleus, where it activates thrombin-responsive genes such as PDGF. It appears likely that this novel mechanism of mRNA-tethering and proteolytic release of a transcription factor will provide a paradigm for the regulation of many transcription factors that have RNA-binding activity. Moreover, release of these proteins from specific mRNAs may regulate their translation, consistent with long-standing suggestions that Y-box proteins play such a role. The findings of DiCorleto's group thus may have killed two birds with one stone: proteolytic cleavage of dbpB regulates transcription of genes in the nucleus and translation of mRNAs in the cytoplasm (Stenina et al., 2000, 2001)!

Signaling from the plasma membrane – tethering and intramembrane proteolysis

The importance of nuclear entry, per se

Sanjay Pimplikar (Case Western Reserve University) described experiments which show that the absence of PAT1 protein from the nucleus initiates apoptosis. PAT1 was identified in a yeast two-hybrid screen using the cytoplasmic tail of Aβ precursor protein (APP) (Zheng et al., 1998). Subsequent studies showed it to be a putative oncogene that is overexpressed in several carcinomas (Monni et al., 2001). PAT1 is normally present both in the nucleus and cytoplasm.

Expression of a PAT1 truncation mutant that is unable to enter the nucleus causes cells to undergo apoptosis. Since these cells also express endogenous full-length PAT1, these data suggest that the truncation mutant acts as a dominant negative, possibly by preventing the nuclear access of an unknown factor. Interestingly, induction of apoptosis by staurosporine also resulted in rapid degradation of PAT1. Although the mechanism by which PAT1 controls cell growth is not known, it is evident that cytoplasmic restriction of this protein is critical.

Notch signaling in non-neuronal cells

The cell surface transmembrane protein, Notch, has been implicated in multiple signaling events. In each case, signaling requires its cleavage by presenilin/γ-secretase, which releases a cytoplasmic fragment (NICD) to the cytosol, which can then enter the nucleus. Before delving into the role of NICD in kidney and hair follicle development, Rafi Kopan (Washington University) raised an important issue: More than a dozen proteins can be cleaved by presenilin/γ-secretase. Are the fragments which are released necessarily active in signaling? Kopan argued that, unlike the story of Notch and possibly CD44, this may not always be the case.

To exemplify the breadth of importance of Notch signaling, Kopan described the use of an antibody which detects the presenilin-cleavage product in the nuclei of cells in brain, kidney and skin. The antibody was used in conjunction with a γ-secretase inhibitor (DAPT), Notch knock-out and presenilin knock-out mice. In kidney, notch signaling is critical for "specifying" that cells become podocytes; however, in its absence, the same cells can progress to a later state of differentiation and become proximal tubule cells. He also presented data to show the involvement of Notch signaling in hair follicle and T-cell development. In skin, Notch signaling is required to maintain appendage differentiation. In both cases, loss of Notch signaling can account for loss of γ-secretase.

The changing roles of APP

Like Notch, APP is also cleaved by presenilin/γ-secretase complex and a number of groups have shown that the resulting cytoplasmic fragment (AICD) (~ 5 kDa) can enter the nucleus and function in signaling and transcriptional activation analogous to NICD, using heterologous systems. Edward Koo (University of California, San Diego) summarized work in this area. Ongoing studies have suggested that cleavage of APP per se to generate the Aβ fragment contributes to cytotoxicity observed in Alzheimer's disease, but the downstream signaling pathway and causes of Alzheimer's Disease pathogenesis remain unclear. The transcriptional activation events have been ascribed to functional interactions of NICD with the Fe65-Tip60 complex (Cao and Südhof, 2001) which ultimately removes the N-COR repressor from chromatin, leading to activation of KAI1 and other genes (Baek et al., 2002). Nevertheless, a recent study from the Südhof group (Cao and Südhof, 2004), suggests that the C-terminal fragment of APP actually may not have to enter the nucleus (it can function even when carrying an NES). Strikingly, even a truncated version of APP in which the cytosolic domain is anchored by a foreign transmembrane domain can do the job. Taken together, this still-evolving story argues strongly in favor of a role for AICD in signaling and transcription.

Koo also described data to show Aβ peptides are able to induce dimerization of holo-APP, which triggers caspase 8 cleavage of the APP cytoplasmic tail to yield a fragment (C31), which heightens the susceptibility of cells to tamoxifen. AICD and the C31 fragment also activate GSK-3β presumably via Fe65. Since GSK-3β phosphorylates Tau, which invariably accumulates in Alzheimer neurons, AICD, or a fragment of AICD, could provide an additional connection between APP metabolism and Tau phosphorylation (Koo, 2002; Kang et al., 2002; Galvan et al., 2002; Soriano et al., 2001).

Signaling from the plasma membrane independent of proteolysis

The tumor suppressor, E-cadherin

Barry Gumbiner (University of Virginia) explained that cell surface E-cadherin has been implicated in tissue formation, cell rearrangements and cell-to-cell communication. Loss of E-

cadherin expression correlates with invasion and metastasis. The cytosolic domain of E-cadherin binds catenins (β-catenin, α-catenin, p120^cat) and (indirectly) to the actin cytoskeleton. β-Catenin is also among the downstream effectors which receive input from Wnt interaction with frizzled at the cell surface. Wnt stimulation acts through a complex of the APC tumor suppressor protein, axin and GSK3β to stabilize β-catenin and allow it to accumulate in the cytosol. Soluble β-catenin is then able to enter the nucleus in conjunction with the HMG-box protein, TCF. These effects are opposed by excess E-cadherin, which can act as a tumor suppressor. The targeting of β-catenin to either the cadherin complex or the TCF transcription complex may be regulated, because protein-protein interaction studies document the existence of multiple forms of modified β-catenin in the cytosol that bind either E-cadherin or TCF (Gottardi et al., 2001) (Gottardi and Gumbiner, unpublished).

The mechanism by which E-cadherin acts as a tumor suppressor has been under dispute. This activity may involve either changes in cell-cell adhesion and/or modulation of β-catenin nuclear entry and downstream transcriptional consequences. In an attempt to distinguish between these possibilities, the Gumbiner group has expressed mutants of E-cadherin which lack either the ability to promote adhesion or the ability to bind β-catenin. In the context of tumor cell invasion assays, β-catenin binding rather than changes in cell adhesion appears critical, thereby emphasizing the importance of nucleocytoplasmic communication per se. Therefore E-cadherin is not only a mechanical cell adhesion protein but rather a signaling receptor whose function is to tell an epithelial cell whether or not it is touching neighboring epithelial cells.

Attempts to identify the mechanism of nuclear entry of β-catenin show that conventional import pathways are not required, although – like importinα – β-catenin does have an armadillo repeat structure (Yokoya et al., 1999; Fagotto et al., 1998). It thus remains an open question as to whether the import of free cytosolic β-catenin can be regulated.

Conclusions, implications, questions

By housing the genome in an organelle, eukaryotic cells cause chromatin to be exposed to an environment of distinct macromolecular composition. The most fundamental consequence of this spatial segregation is the requirement for machinery to export transcripts to the translational apparatus in the cytoplasm. In addition, spatial segregation provides opportunities for regulating the titer of factors which modulate transcription, covalent processing of transcripts, and their export per se.

Present information indicates that the major thoroughfares for continuous nucleocytoplasmic transport of soluble macromolecules larger than 40–50 kDa are governed by (*1*) Ran in conjunction with members of the importin/exportin family of proteins, and (*2*) the distinct equipment which exports most mRNAs.

Various cargoes can be bridged to this classical equipment by adaptors, whose ability to recognize cargo and adaptors may also be regulated. Moreover, the transport repertoire of these major pathways can include proteins which are so small that one would expect them to be able to diffuse across the NPC, e.g.

histones. The import of the small cytoplasmic domains of Notch and APP may therefore also be regulated.

Aspects of nucleocytoplasmic transport nevertheless remain very obscure. Thus, some cargoes simply do not require the classical equipment (Xu and Massague, 2004) and transport pathways are often perturbed upon viral infection. Moreover, as an extreme example of regulation, the macro- and micronuclei of *Tetrahymena* both have nuclear pores, but do not show parallel behavior with regard to import (White et al., 1989). Among other issues related to the subject matter of the Symposium is the question of whether transport factors associate with latent cargoes prior to their being released from tethers. Moreover, at the most fundamental level, one cannot help but wonder why the nucleus is in fact housed within elements of the ER rather than being surrounded by an altogether distinct type of membrane. Perhaps the presence of Sec13 as a component of both the NPC and ER exit sites reflects a distant evolutionary link between these two "ER specializations."

The intimate enclosure of the genome within the ER would seem to offer unparalleled opportunities for communication between the ER lumen and the nucleoplasm. Although there is very little information concerning such events, the present catalogue of proteins which selectively localize to the nuclear perimeter includes many which are associated with disease (Schirmer et al., 2003). Analysis of these candidates – along with further exploration of the process by which membrane proteins are delivered via "lateral channels" of the NPC from the ER at large to the INM – may help identify such signals.

The Symposium repeatedly emphasized an insistence on understanding mechanism, and forcefully emphasized the cell biological parallels between the multiple systems, cell types and diseases with which individual investigators are concerned.

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