Cell biology
Paper alert

A selection of interesting papers that were published in the two months before our press date in major journals most likely to report significant results in cell biology.

Current Opinion in Cell Biology 2000, **12**:645–652

Contents (chosen by)

- • of special interest
- •• of outstanding interest

Cytoskeleton

Selected by Arshad Desai
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The *Xenopus* chromokinesin Xkid is essential for metaphase chromosome alignment and must be degraded to allow anaphase chromosome movement. Funabiki H, Murray AW: *Cell* 2000, **102**:411-424.

AND

Xkid, a chromokinesin required for chromosome alignment on the metaphase plate. Antonio C, Ferby I, Wilhelm H, Jones M, Karsenti E, Nebreda AR, Vernos I: *Cell* 2000, **102**:425-435.

•• **Significance**: This is the first clear evidence for the involvement of a chromosome-arm-associated kinesin in chromosome alignment.

Findings: Funabiki and Murray identified the XKid chromokinesin using a biochemical assay for chromosome-associated proteins degraded at the metaphase–anaphase transition. Antonio *et al.* identified the same kinesin in a screen for mRNAs differentially associated with polysomes after progesterone treatment of frog oocytes. Both groups found that XKid localizes to chromosomes, is required for chromosome alignment in spindles assembled in frog egg extracts and gets progressively degraded as the extract spindles undergo anaphase. Both groups also found that misalignment occurs because chromosome arms fail to congress to the metaphase plate. Antonio *et al.* show that XKid activity is required for establishment as well as maintenance of chromosome alignment. Funabiki and Murray also demonstrate that XKid is degraded by ubiquitin-mediated proteolysis and use non-degradable versions of XKid to establish that XKid degradation is necessary for anaphase chromosome movement in *Xenopus* extract spindles.

CLIP170-like tip1p spatially organizes microtubular dynamics in fission yeast. Brunner D, Nurse P: *Cell* 2000, **102**:695-704.

• **Significance**: A CLIP170-like microtubule-end-binding protein is required to guide microtubules along the long axis of the fission yeast *S. pombe* and thereby correctly position growth sites at cell ends.

Findings: tip1p, a fission yeast member of the CLIP170 family, was identified in a screen for genes whose overexpression caused aberrant cell morphologies. Deletions of *tip1* are viable but do not localize the cell-end marker Tea1p to the correct location and have shorter microtubules. Live imaging of microtubules showed that deletion of *tip1* did not alter the growth rate but result in increased transitions from growth to shrinkage (catastrophes). In *S. pombe* interphase cells, catastrophes occur predominantly when microtubules encounter the cortex. In wild-type cells microtubules that encounter the cortex near the middle of the cell continue growing until they reach the cell ends. In *tip1* deletions, microtubules undergo a catastrophe fairly soon after they encounter the cortex in the middle of the cell, suggesting that tip1p protects microtubules from cortex-stimulated catastrophes in the middle regions of the cell, thereby guiding microtubules to cell ends and localizing growth to the ends.

Kinetochore reproduction in animal evolution: Cell biological explanation of karyotypic fission theory. Kolnicki RL: *Proc Natl Acad Sci USA* 2000, **97**:9493-9497.

• **Significance**: Kolnicki postulates a mechanism that may account for the origins of karyotypic variability between related species.

Findings: The concept of reciprocal ('Robertsonian') translocations has dominated discussions of the origins of varying chromosome numbers between related species. In 1970, Todd postulated the simultaneous 'fission' of a large number of chromosomes as an alternate mechanism (termed karyotypic fission theory; KFT). KFT has been generally ignored because of the lack of any mechanistic underpinnings. The author presents a speculative mechanism for KFT, where dicentric chromosomes are formed en masse as a consequence of a rare centromere duplication event, generating monocentric chromatids with dicentric sisters. During meiosis II, the attachment of both centromeres of the dicentric sister to the same pole is necessary to bypass the tension-sensitive spindle checkpoint, favoring generation of gametes with dicentric chromatids. Replication of the dicentric chromatid after fertilization would generate a chromosome with four kinetochores, a structure that is likely to undergo double-stranded breaks generating 'fissioned' chromosomes. This hypothesis represents an interesting mechanistic speculation of how karyotypic fissioning may occur.

HIV-1 Rev depolymerizes microtubules to form stable bilayered rings. Watts NR, Sackett DL, Ward RD, Miller MW, Wingfield PT, Stahl SS, Steven AC: *J Cell Biol* 2000, **150**:349-360.

• **Significance**: The HIV-1 Rev protein acts as a microtubule destabilizer.

Findings: One consequence of HIV-1 infection is massive cytoskeletal reorganization. The HIV-1 encoded regulator Rev has primarily been studied in the context of its role in exporting viral RNAs out of the nucleus. In this paper, *in vitro* evidence is presented showing that Rev can depolymerize microtubules into stable rings. Structural analysis revealed that the rings are bilayered and have equimolar Rev and tubulin subunits. At low Rev:Tubulin stoichiometries, peeling protofilaments that convert into ring-like structures are observed at both microtubule ends.

Furthermore, a segment of Rev exhibits sequence similarity to a part of the catalytic domain of the microtubule-destabilizing Kin I kinesins. Rev also prevents microtubule assembly in *Xenopus* egg extracts. These results suggest that in addition to its role in RNA export, Rev may influence cellular structure by depolymerizing microtubules in HIV-1 infected cells.

Selected by Elizabeth A Holleran
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p53 is associated with cellular microtubules and is transported to the nucleus by dynein. Giannakakou P, Sackett DL, Ward Y, Webster KR, Blagosklonny MV, Fojo T: *Nat Cell Biol* 2000, **2**:709-717.

•• **Significance**: Inactivation of the tumor suppressor protein p53 is the most common alteration in human cancers. Although an abundance of information exists to describe its relation to apoptosis and its function in growth arrest, less is known about the cytoplasmic accumulation and exclusion of p53 from the nucleus that results in its inactivation. Here, an integral link between the microtubule system and dynein motor protein provides insight into the mechanism of p53 transport and accumulation in the nucleus of cells after DNA damage.

Findings: Microtubule copelleting assays and immunoprecipitations using antibodies to tubulin and to p53 reveal that p53 directly associates with the microtubule cytoskeleton. In fixed cells, confocal microscopy reveals colocalization of p53 with cellular microtubules and accumulation of p53 in the nucleus after DNA damage. Disruption of the microtubule cytoskeleton impairs p53 accumulation in the nucleus after DNA damage. Reagents that have been previously described to inhibit the minus-end-directed motor protein, dynein, inhibit nuclear accumulation of p53 after DNA damage. Exogenous microtubules were added to extracts in the presence of AMP-PNP to promote motor protein binding. More p53 was detected in AMP-PNP treated extracts than with just ATP, suggesting the association of p53 with a motor protein. Additional findings revealed that p53 coprecipitates with the p50 subunit of dynactin and dynein using antibodies to each of these proteins.

Dynamic relocalization of histone macroH2A1 from centrosomes to inactive X chromosomes during X inactivation. Rasmussen TP, Mastrangelo M-A, Eden A, Pehrson JR, Jaenisch R: *J Cell Biol* 2000, **150**:1189-1198.

•• **Significance**: MacroH2A1 is a histone protein variant that has been previously localized on inactive X chromosomes. Here, 95% of the centrosomes from undifferentiated female and male embryonic stem (ES) cells contain a nonchromatin-associated population of MacroH2A1. Accumulation of MacroH2A1 on X chromosomes corresponded with a loss of MacroH2A1 on centrosomes. These results describe a potential pathway for delivery and loading of this unique histone onto the inactive X chromosome during differentiation and suggest a role for the centrosome in X inactivation.

Findings: In undifferentiated ES cells, MacroH2A1 accumulates around the centrosomes. Histone and chromatin are not typically thought to be directly associated with centrosomes. Affinity purified antibodies to MacroH2A1 detect the protein in fractions of purified centrosomes and in cultured cells. MacroH2A1 associates with centrosomes in a microtubule-dependent manner, as demonstrated by the use of nocodozole. MacroH2A1 also cosedimented with γ-tubulin and Skp1 components of the centrosome. Potential models for MacroH2A1 association with centrosomes in undifferentiated ES cells include a yet undescribed centrosomal function of MacroH2A. More likely, centrosomes may serve as a storage site for MacroH2A1 before its use during differentiation. The mechanism of MacroH2A transport from the centrosome to the inactive X chromosome remains to be determined.

The deaf jerker mouse has a mutation in the gene encoding the epsin actin-bundling proteins of hair cell stereocilia and lacks epsins. Zheng L, Sekerkova G, Vranich K, Tilney LG, Mugnaini E, Bartles JR: *Cell* 2000, **102**:377-385.

•• **Significance**: Epsins (actin-bundling proteins) have been previously described in brush border microvilli and Sertoli cell-spermatid junctions. This paper reports the discovery of epsins in hair cell stereocilia. *epsin* maps to chromosome 4 at the same region as *jerker*, a recessive mutation that causes deafness, vestibular dysfunction and hair cell degeneration. The phenotype in jerker mice is caused by a mutation in *epsin*.

Findings: To detect sound as well as motion, hair cells use stereocilia as mechanoelectrical transducers. Each stereocillium in a hair cell consists of a projection of the apical plasma membrane containing parallel bundles of actin filaments. Stereocilium are linked to one another in adjacent rows and stretch open ion channels resulting in hair cell depolarization. The identity of actin-bundling proteins involved in this process has remained elusive. Here, the *epsin* gene has been found in sterocilia and appears to be the gene mutated in mice cells that have the jerker phenotype. These mice have normal levels of epsin mRNA but do not accumulate the protein in their tissues. The molecular defect arises from a frameshift mutation that destroys the carboxy-terminal actin-bundling portion of epsin and possibly effects the stability of epsin protein.

Cell regulation

Selected by Serge Roche
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Src tyrosine kinase is a novel direct effector of G proteins. Ma Y-C, Huang J, Ali S, Lowry W, Huang XY: *Cell* 2000, **102**:635-646.

•• **Significance**: A new mechanism by which receptor-coupled G proteins induce tyrosine phosphorylation has now been uncovered: Gα subunits physically interact with and activate the cytoplasmic tyrosine kinase Src. This may explain why Gα proteins are oncogenic in tumours.

Findings: Gαs and Gαi, but not Gβγ nor Gαq, directly activate the cytoplasmic tyrosine kinases Src and Hck *in vitro*. The α subunit stimulates enzymatic activity by physical contact with the catalytic domain, leading to a conformational change that allows easier access of the substrate. Interestingly, this process does not require dephosphorylation of the negative regulatory tyrosine, Y527 (in chicken Src). Gα protein mutants defective in interacting with Src failed to activate any tyrosine kinases *in vivo*, indicating that Src is an essential mediator for Gα-induced protein tyrosine phosphorylation.

The Syk tyrosine kinase suppresses malignant growth of human breast cancer cells. Coopman PJ, Do MT, Barth M, Bowden ET, Hayes AJ, Basyuk E, Blancato JK, Vezza PR, McLeskey SW, Mangeat PH, Mueller SC: *Nature* 2000, **406**:742-747.

•• **Significance**: The haematopoietic cytoplasmic tyrosine kinase Syk is a potential tumour suppressor in human breast cancer.

Findings: Although originally thought of as haematopoietic in origin, Syk is also expressed in breast epithelial cells.

Surprisingly, its expression was downregulated in breast cancer cells, suggesting a negative function for this kinase during carcinogenesis. Indeed, reintroduction of Syk to Syk-deficient cells reduced malignant growth, whereas expression of a kinase dead mutant in Syk positive cells enhanced their tumorigenic effect. The negative function of Syk was attributed to an alteration in mitosis and cytokinesis. Whether Syk acts as a similar tumour suppresser activity in other carcinoma was not addressed.

Site-specific serine phosphorylation of the IL-3 receptor is required for hematopoietic cell survival. Guthridge MA, Stomski FC, Barry EF, Winnall W, Woodcock JM, McClure BJ, Dottore M, Berndt MC, Lopez AF: *Mol Cell* 2000, **6**:99-108.
• **Significance**: Although cytokine receptors do not possess any intrinsic catalytic activity, they signal through cytoplasmic tyrosine kinases for receptor tyrosine phosphorylation, which leads to recruitment of signalling molecules posessing Src homology and/or phosphotyrosine-binding domains. An alternative route has been now identified where serine phosphorylation is used for such signalling events.
Findings: The interleukin 3 (IL-3) receptor mutant, where the eight tyrosines present in the cytoplasmic tail were replaced by phenylalanine, still allows haematopoietic cell survival. Therefore, an alternative, and to date unknown mechanism, of signalling had been proposed. The authors now report that Ser585 of the cytokine common βc receptor subunit is phosphorylated upon appropriate ligand binding. Phosphorylated Ser585 creates a binding site for the 14-3-3 family of proteins, which bridge the survival enzyme phosphoinositide 3-kinase (PI3K) and the receptor. Evidence suggests that protein kinase A is the responsible kinase. The importance of this new signalling pathway is underscored by the inability of the mutated IL3 receptor to associate with PI3K and promote cell survival.

The EGF receptor provides an essential survival signal for SOS-dependent skin tumor development. Sibilia M, Fleischmann A, Behrens A, Stingl L, Carroll J, Watt FM, Schlessinger J, Wagner EF: *Cell* 2000, **102**:211-220.
• **Significance**: The EGF receptor has an essential role in cell survival in oncogenic transformation.
Findings: The EGF receptor and Ras are frequently deregulated in human epithelial tumours; however, their exact role in this process has not been established. To address this question, transgenic mice were generated that specifically express a constitutive active form of the Ras activator Son of Sevenless (SOS-F) in the skin. Mice strains develop spontaneous skin tumours, which were abolished in transgenic mice with an $EGFR^{-/-}$ or hypomorphic background. SOS-F oncogenic activity was also EGF-receptor-dependent in immortalized fibroblasts. This effect was attributed to increased cell survival and not to enhanced cell proliferation.

The protooncogene TCL1 is an Akt kinase coactivator. Laine J, Kunstle G, Obata T, Sha M, Noguchi M: *Mol Cell* 2000, **6**:395-407.
• **Significance**: This report identifies a new mechanism for activation of the survival kinase Akt and provides a molecular mechanism by which the TCL1 protooncogenes promote oncogenic cell transformation.
Findings: TCL1 is a member of the protooncogene family upregulated in prolymphocytic leukaemia and which encodes a protein with unknown function(s). The authors now provide strong evidence for TCL1 as a coactivator of Akt. The kinase interacts with TCL1 in a yeast genetic two-hybrid assay, and TCL1 and its isoforms bind to the pleckstrin homology domain of Akt, leading to increase in kinase activity. TCL1 forms a trimer, thus facilitating oligomerization, phosphorylation and ultimately activation of Akt. Finally, expression of TCL1 in cells enhanced cell proliferation and survival.

Myotubularin, a protein tyrosine phosphatase mutated in myotubular myopathy, dephosphorylates the lipid second messenger, phosphatidylinositol 3-phosphate. Taylor GS, Maehama T, Dixon JE*: Proc Natl Acad Sci USA* 2000, **97**:8910-8915.
•• **Significance**: The second messenger phosphatidylinositol 3-phosphate (PI3P) is involved in intracellular vesicular trafficking and binds to protein with FYVE homology domains. This signalling molecule has now been linked to human disease.
Findings: *MTM1* is mutated in X-linked myotubular myopathy, which displays an impairment in myogenic differentiation. *MTM1* encodes the protein myotubularin, which has sequence similarity with dual phosphatase proteins. Myotubularin is now shown to be a specific phosphatidylinositol 3-phosphatase. Products of *MTM1* mutations found with severe myotubular myopathy have impaired phosphatase activity, indicating that an excess of PI3P has important consequences for myogenic differentiation. Interestingly, other myotubularin-like proteins have been found in protein databases, suggesting that it belongs to a family of PI3P phosphatases.

Selected by Orion Weiner
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NO is necessary and sufficient for egg activation at fertilization. Kuo RC, Baxter GT, Thompson SH, Stricker SA, Patton C, Bonaventura JB, Epel D: *Nature* 2000, **406**:633-636.
•• **Significance**: Early events in egg activation were previously unknown. This report demonstrates that nitric oxide (NO) is both necessary and sufficient for egg activation. As NO is also present in the appropriate spatial and temporal distributions for egg activation, these data strongly suggest that NO is an egg activator.
Findings: Using sea urchin gametes as their experimental system, the authors found that NO activity is present in sperm during the acrosome reaction and in eggs at fertilization. Increasing intracellular NO induced egg activation, and preventing NO production through the use of oxyHb, prevented egg activation, although it did not prevent sperm from entering the cells. On the basis of these data, the authors suggest that NO may be a universal activator of eggs or oocytes.

Two pathways through Cdc42 couple the N-formyl receptor to actin nucleation in permeabilized human neutrophils. Glogauer M, Hartwig J, Stossel T: *J Cell Biol* 2000, **150**:785-796.
• **Significance**: This is the first detailed dissection of the signal transduction cascade involved in chemoattractant-mediated actin polymerization in a biochemically accessible permeabilized neutrophil system.
Findings: Permeabilized cell systems provide a powerful approach to dissecting signal transduction cascades in a biochemically accessible fashion. This approach had previously been used to demonstrate that Rac-induced phospholipid production is critical for actin polymerization during platelet activation. The authors now extend this approach to investigate actin polymerization in permeabilized neutrophils. They find that actin polymerization induced by chemoattractant requires Cdc42. Downstream of Cdc42 two diverging pathways were found to interface with the actin cytoskeleton. One requires Rac activation,

the other Arp2/3 complex activation. Both pathways depend on phospholipids, although whether phospholipids act solely through uncapping filament ends or whether they also activate N-WASP or other pathways to the actin cytoskeleton is not yet clear.

Tenascin-C suppresses Rho activation. Wenk MB, Midwood S, Schwarzbauer JE: *J Cell Biol* 2000, **150**:913-919.
• **Significance**: A novel example of cytoskeletal regulation by the extracellular matrix is presented that involves downregulation of the GTPase Rho.
Findings: Changes in tissue organization during development, disease and injury involve changes in the organization of the extracellular matrix. Adhesion properties of cells during these processes are modulated through expression of the adhesive protein fibronectin and the anti-adhesive protein tenascin-C, which modulates cell–fibronectin interactions. Using an activation assay for the Rho GTPases, and modulation of Rho GTPase activity, the authors find that tenascin-C suppresses stress fibers and induces actin-rich filopodia for cells grown on fibronectin. These morphological changes are correlated with a complete suppression of RhoA activity (a small GTPase involved in myosin regulation and cell contractility). Activation of RhoA blocked the effects of tenascin and inactivation of Rho phenocopies tenascin. These results suggest that tenascin induces its morphological effects on the cytoskeleton through suppression of Rho activity.

Nucleus and gene expression

Selected by Rein Aasland
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Regulation of chromatin structure by site-specific histone H3 methyltransferases. Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun Z-W, Schmid M, Opravil S, Mechtker K, Ponting CP, Allis CD, Jenuwin T: *Nature* 2000, **406**:593-599.
•• **Significance**: The role of acetylation of the nucleosomal histone tails transcriptional regulation has been extensively studied. Much less is known about other post-translational histone modifications such as methylation and phosphorylation. Here, the SET domain, an evolutionarily conserved domain found in a number of proteins implicated in epigenetic gene regulation, is found to have a novel type of histone methyltransferase activity.
Findings: Sensitive database searches identified a weak similarity between the SET domain and certain plant methyltransferases. The bacterially expressed SET domains of homologues of Su(var)3-9 (a protein encoded by a *Drosophila* suppressor of position effect variegation) was found to have histone methyltransferase activity *in vitro*. Lys9 in the amino-terminal tail of histone H3 was the only substrate.

A link between RNA interference and nonsense-mediated decay in *Caenorhabditis elegans*. Domeier ME, Morse DP, Knight SW, Portereiko M, Bass BL, Mango SE: *Science* 2000, **289**:1928-1930.
• **Significance**: RNA interference (RNAi) is a type of post-transcriptional gene silencing where double-stranded RNA molecules elicit degradation of cognate endogenous mRNAs. In many cases, the RNAi effect can spread in the organism and persist through many cell generations. In the present report, a functional link is established between RNAi and nonsense-mediated mRNA decay (NMD). NMD prevents mutant mRNAs from serving as templates for synthesis of truncated proteins.
Findings: Three genes (*smg-2, -5,* and *-6*) that are required for NMD in *C. elegans* were also found to be necessary for RNAi. In the *smg* mutants, the RNAi effect was initially normal but ceased after a couple of days, suggesting that the three *smg* genes are involved in the maintenance of RNAi.

Identification of a regulated pathway for nuclear pre-mRNA turnover. Bousquet-Antonelli C, Presutti C, Tollervey D: *Cell* 2000, **102**:765-775.
• **Significance**: The exosome is a multi-protein complex responsible for removal of cytoplasmic mRNA as well as for processing and degradation of nuclear RNAs such as rRNA and small nucleolar RNAs. It has previously been proposed that a degradation pathway for nuclear unspliced pre-mRNAs may exist. In the present paper, it is shown that nuclear pre-mRNAs are degraded from both their 5′ and 3′ ends by the Rat1p exonuclease and the nuclear variant of the yeast exosome, respectively. It is proposed that nuclear pre-mRNA degradation in mammalian cells will be even more significant, as alternative splicing is extensively used in these organisms.
Findings: In yeast strains mutant for the essential exosome component Rrp41p, the levels of pre-mRNA from all genes tested accumulated. In strains deficient in cytoplasmic mRNA degradation, the tested pre-mRNAs did not accumulate, strongly supporting the idea that pre-mRNA degradation takes place in the nucleus. It appears that there is completion between degradation and splicing. Interestingly, pre-mRNA degradation appeared to be subject to regulation by the availability of glucose.

Selected by Robert OJ Weinzierl,
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Chromatin fine structure profiles for a developmentally regulated gene: reorganization of the lysozyme locus before trans-activator binding and gene expression. Kontaraki J, Chen H-H, Riggs A, Bonifer C: *Genes Dev* 2000, **14**:2106-2122.
•• **Significance**: The chicken lysozyme gene is one of the best-characterized model systems for studying the influence of different chromatin configurations on transcriptional activity. This study describes the application of new and very sensitive techniques to assay changes in chromatin states at various developmental stages. The evidence presented suggests that the developmental 'maturation' of specific chromatin stages probably occurs independently of the presence of gene-specific transcription factors.
Findings: Chromatin fine structure and transcription factor occupancy of the 'early' enhancer and promoter of the chicken lysozyme gene were examined at single nucleotide resolution at various stages of macrophage differentiation. A novel quantitative UV photofootprinting technique, coupled with the recently developed 'terminal transferase-dependent PCR (TDPCR)' allowed the sensitive detection of all structural changes in DNA that are induced by the localized binding of transcription factors and/or nucleosomes. There is clear evidence for the establishment of an 'active' chromatin architecture in early multipotent precursor cells that do not yet transcribe lysozyme, thus setting the stage for active transcription of the gene once the appropriate gene-specific transcription factors are expressed in the correct combinations.

Phosphorylation of linker histone H1 regulates gene expression in vivo by creating a charge patch. Dou Y, Gorovsky MA: *Mol Cell* 2000, **6**:225-231.
• **Significance**: The overall functional role of linker histones, such as H1, and their structural involvement in the various chromatin environments is still poorly understood. This paper suggests that the creation of negative charge patches

through differential phosphorylation regulates some of the biological properties of H1.
Findings: Disruptions of the genes encoding histone H1 in various organisms cause only relatively minor expression defects, which are restricted to a small number of nonessential genes and thus do not affect overall viability. In this study, the authors extensively mutagenised the region of H1 from *Tetrahymena* that is normally subject to regulated phosphorylation. The results show that any changes making the region more negatively charged, even in residues that are normally not subject to phosphorylation, can mimic the overall effect of phosphorylated H1 *in vivo*. The localized increase in negative charge may cause a conformational change in H1, affect the nonspecific DNA-binding properties, and/or increase the access of transcription factors to DNA.

Molecular structure of human TFIIH. Schultz P, Fribourg S, Poterszman A, Mallouh V, Moras D, Egly JM: *Cell* 2000, **102**:599-607.

AND

Electron crystal structure of the transcription factor and DNA repair complex, core TFIIH. Chang W-H, Kornberg RD: *Cell* 2000, **102**: 609-613.
•• **Significance**: TFIIH is an enzymatically active multiprotein complex playing multiple key roles in RNA polymerase II transcription and nucleotide-excision repair. The two studies provide the first structural insights into the overall molecular shape of TFIIH from two different eukaryotic species.
Findings: The structure of human holo-TFIIH was determined to low resolution (38 Å) by single particle cryoelectron microscopy, whereas the medium resolution structure (18 Å) structure of yeast core TFIIH was derived from electron diffraction of two-dimensional crystals in charged lipid bilayers. Both structures show a ring-like structure that is characteristic of several other enzyme complexes involved in DNA replication, repair and recombination. Comparison of core- and holo-TFIIH, together with immunolabelling experiments with antibodies directed against specific subunits, allowed the placement of several key subunits, such as the cyclin-dependent kinase cdk7 and the DNA-helicase XPD and XPB.

Membranes and sorting

Selected by Vas Ponnambalam
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Aggregation chaperones enhance aggregation and storage of secretory proteins in endocrine cells. Jain RK, Joyce PBM, Gorr SU: *J Biol Chem* 2000, **275**:27032-27036.
• **Significance**: Calcium-induced aggregation has been proposed to play a role in the sorting and storage of proteins in dense-core secretory granules in higher eukaryotes. Low pH and millimolar calcium concentrations are key factors in this aggregation model, although the exact mechanism by which this occurs *in vivo* is not known. Failure to identify 'receptors' that mediate aggregation or protein sorting has led to a variety of hypotheses linked to the aggregation properties of individual hormones and other cargo proteins. In this study, the authors show that a secretory protein can influence the efficiency of aggregation of a regulated secretory protein *in vivo* and *in vitro*, thus acting as an aggregation chaperone.
Findings: Hexahistidine-tagged secretory proteins stimulate the calcium-induced aggregation of pro-enkaphalin and chromogranin A *in vitro*. Importantly, a hexahistidine-tagged alkaline phosphatase continues to be constitutively secreted but stimulates the efficiency of chromogranin A incorporation into dense-core secretory granules. Thus, the proportion of chromogranin A directed into the regulated secretory pathway was increased by 50% in the presence of a hexahistidine-tagged alkaline phosphatase.

Anterograde flow of cargo across the Golgi stack potentially mediated via bidirectional 'percolating' COPI vesicles. Orci L, Ravazzola M, Volchuk A, Engel T, Gmachl M, Amherdt M, Perrelet A, Sollner T, Rothman JE: *Proc Natl Acad Sci USA* 2000, **97**:10400-10405.
• **Significance**: There is much controversy as to the exact mechanism by which newly synthesised proteins traverse the Golgi stack before reaching the cell surface. Two different models that invoke vesicular transport and cisternal progression are the focus of current studies. Although it is generally accepted that COPI-coated transport vesicles mediate retrograde (or backwards) transport through the Golgi stack and back to the ER, the role of these vesicles in anterograde (or forward) transport is unclear. Orci *et al.* show that at least two different populations of COPI-coated vesicles exist and may mediate these two different transport routes through the Golgi stack.
Findings: Quantitative immunoelectron microscopy shows that the GOS-28/HA-tagged synatxin 5 SNARE complex is present throughout the Golgi stack; however, GOS-28 is absent from KDEL receptor-containing COPI vesicles associated with the Golgi stack and involved in retrograde traffic. Analysis of a constitutively secreted glycoprotein, such as the vesicular stomatitis G protein, reveals colocalisation with GOS-28 in COPI vesicles pointing to an anterograde transport route.

The luminal domain of TGN38 interacts with integrin β1 and is involved in its trafficking. Wang J, Howell K: *Traffic* 2000, **1**:713-723.
•• **Significance**: A key area in cell biology is the identification and characterisation of 'cargo' receptors that mediate the vectorial movement of soluble and membrane-bound proteins between two intracellular compartments. A variety of signals displayed within the lumen of a compartment may be recognised by different classes of receptors. For example, different types of N-linked sugars on secretory proteins are recognised by mammalian lectins such as VIP36 or ERGIC-53. However, identification of other 'cargo' receptors to account for the large volume of membrane traffic has remained elusive. In this report, the authors show that a ubiquitously expressed trans-Golgi network resident (TGN38) binds to the β1 integrin and may control its secretion to the cell surface.
Findings: Transfection and expression of a soluble TGN38 lumenal domain fragment caused cell detachment and association of this soluble protein with cellular 'footprints'. This soluble fragment was associated with the α5β1 integrin and stimulated cell surface integrin levels. Endogenous TGN38 was associated with a small but reproducible fraction of intracellular β1, indicating that it regulates the trafficking of newly synthesised β1 integrin along the secretory pathway.

Mutations in synaptojanin disrupt synaptic vesicle recycling. Harris TW, Hartweig E, Horvitz HR, Jorgensen EM: *J Cell Biol* 2000,**150**:589-599.
• **Significance**: Membrane recycling and turnover at the cell surface is dependent on the endocytic pathway. During neurotransmission, synaptic vesicle recycling is postulated to occur either via a kiss-and-run mechanism or by recovery via the endocytic route. Synaptojanin is a polyphosphoinositide

phosphatase that is found at synapses and implicated in regulating endocytosis. These authors show that the *C. elegans unc-26* gene is the ortholog of mammalian synaptojanin and *unc-26* mutants display phenotypes consistent with defects in synaptic vesicle recycling.
Findings: The *C. elegans* synaptojanin ortholog was mapped to the *unc-26* gene; an RFLP (restriction fragment length polymorphism) is detected in the *unc26*(n1307) mutant by comparison to the wild-type allele. All 13 *unc-26* mutant alleles examined had specific point mutations or changes in the *unc-26* coding region. Electron microscopy on *C. elegans unc-26* mutants showed significant decrease (>50%) in synaptic vesicles at the synapses of GABA-releasing and cholinergic neurons. These cells showed significant accumulation of clathrin-coated pits and vesicles and coated vesicles associated with the Golgi apparatus. *unc-26* mutations also caused increased endosome size; these findings point to a key role for synaptojanin in facilitating endocytosis and synaptic vesicle recovery.

Kaposi's sarcoma-associated herpesvirus encodes two proteins that block cell surface display of MHC Class I chains by enhancing their endocytosis. Coscoy L, Ganem D: *Proc Natl Acad Sci USA* 2000, **97**:8051-8056.
•• **Significance**: Subversion of host defence mechanisms is a key feature of pathogenic infections. The Class I MHC cell surface glycoprotein binds and displays a wide variety of host cell-derived peptides; this is critical for immune surveillance by circulating cytotoxic T lymphocytes (CTLs). If MHC Class I molecules display foreign or viral-derived peptides at the cell surface, CTLs immediately recognise and kill the infected cell. This report shows that a human herpesvirus (KSHV) dramatically reduces cell surface Class I MHC expression by expressing two proteins that stimulate endocytosis and lysosomal degradation of these cell surface molecules.
Findings: Two (K3 and K5) of the fifteen ORFs in KSHV produced strong (approximately 20–30 fold) reduction in cell surface MHC Class I in transfected HeLa cells. Tagged K3 and K5 localise to the endoplasmic reticulum (ER); these patterns do not colocalise with endogenous MHC Class I, which is now redistributed into punctate cytoplasmic vesicles characteristic of the endosome/lysosome system. Metabolic labelling experiments show that synthesis, assembly and transport of MHC Class I in the presence of K3 and K5 is unaffected. However, at later time points MHC Class I levels is significantly reduced via a dynamin1-regulated pathway in K5-expressing cells; this effect could be overcome with lysosomotropic agents or vacoular ATPase inhibitors. Thus K3 and K5 are associated with the ER but can influence endocytic trafficking via a novel mechanism or signalling pathway.

Two distinct regions in a yeast myosin-V tail domain are required for the movement of different cargoes. Catlett NL, Duex JE, Tang F, Weisman LS: *J Cell Biol* 2000, **150**:513-525.
• **Significance**: Molecular motors such as kinesins, dyneins and myosins mediate intracellular movement of vesicles and organelles. One key question is the nature of interaction between the vesicle coat/cargo and a molecular motor. The cytoplasmic domains of transmembrane 'cargo' proteins may bind directly to specific molecular motors; alternatively, these proteins may interact with cytoplasmic vesicle coat proteins that in turn bind to such motors. Catlett *et al.* show that the budding yeast Myo2p motor contains specific sequences that mediate vacuole movement and inheritance and thus move multiple cargoes.
Findings: Random mutagenesis of Myo2p tail domain highlights an 11 residue stretch (1297–1307); point mutations in this region affect vacoule inheritance. A downstream deletion (deletion 1459–1491) does not affect vacuole inheritance but retains segregation structures connecting the mother and daughter bud vacuoles. This truncated allele has properties consistent with retention of the ability to move vacuoles but not essential cargo. These findings point to two different regions on the tails of the Myo2p homodimer binding different cargoes through interaction with organelle-specific receptors.

Membrane permeability

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Receptor-mediated hydrolysis of plasma membrane messenger PIP_2 leads to K^+-current desensitization. Kobrinsky E, Mirshahi T, Zhang H, Jin T, Logothetis DE: *Nat Cell Biol* 2000, **2**:507-514.
•• **Significance**: G-protein-gated inwardly rectifying K^+ channels play an important role in regulating heartbeat. Sustained stimulation of G protein (Gαi)-coupled receptors leads to a rapid activation of GIRK channels followed by a slow decrease in channel activity ('desensitisation'). In this paper, Kobrinsky *et al.* provide evidence that a depletion in PIP_2 (phosphatidylinositol bis-phosphate) levels, caused by activation of Gq-protein-coupled receptors, underlies desensitisation.
Findings: In *Xenopus* oocytes or mammalian cells coexpressing the M1 (Gq) and M2 (Gi) muscarinic receptors, stimulation of the M1 receptor decreased the M2-activated GIRK current. Confocal imaging of GFP-tagged PH (pleckstrin homology) domain of PLCδ was used to monitor translocation of PIP_2. Mutant inward rectifiers that showed weak interactions with PIP_2 were inhibited more strongly following stimulation of Gq-coupled receptors.

Targeting of PKA to glutamate receptors through a MAGUK-AKAP complex. Colledge M, Dean RA, Scott GK, Langeberg LK, Huganir RL, Scott JD: *Neuron* 2000, **27**:107-119.
•• **Significance**: Phosphorylation of glutamate receptors is important for learning and memory. PDZ-containing proteins (PSD95 and SAP97) are known to interact with glutamate receptors. Here, Colledge *et al.* show that kinase anchoring proteins (AKAPs) interact directly with PSD95/SAP97 but not through the PDZ domains.
Findings: Using an A kinase overlay system, AKAP150 (an ortholog of AKAP79) was identified in postsynaptic density (PSD)-enriched membrane fractions. AKAP150/79 colocalised and coimmunoprecipitated with PSD95. AKAP150/79 competed for calcineurin binding to PSD95. Mutagenesis studies revealed that AKAP150/79 interacted via SH_3 and guanylate kinase domains and not through the PDZ domains. Functional studies showed that AKAP150/79 also coprecipitated with glutamate receptors and was involved in channel phosphorylation.

State-dependent cross-inhibition between transmitter-gated cation channels. Khakh BS, Zhou X, Sydes J, Galligan JJ, Lester HA: *Nature* 2000, **406**:405-410.
• **Significance**: Purinergic P2X (ATP-gated) and nicotinic (acetylcholine-gated) receptors are both ligand-gated ion channels but have different membrane topology. Surprisingly, Khakh *et al.* report in this paper that stimulation of $P2X_2$ receptors, which are coexpressed with nicotinic receptors, leads to an inhibition of acetylcholine-gated activity. Although the mechanism

of inhibition is unknown at present, the results suggest P2X and nicotinic receptors interact directly.
Findings: Cross-inhibition was studied in *Xenopus* oocytes coexpressing $P2X_2$ and $\alpha3\beta4$ nicotinic receptors. Interestingly, cross-inhibition was not observed at low expression levels. A mutant $P2X_2$ receptor that desensitised quickly showed transient inhibition of acetylcholine-activated nicotinic receptors. In myenteric neurones, ATP activation of P2X receptors inhibited the fast, acetylcholine-dependent excitatory synaptic potentials.

A voltage-dependent channel involved in nutrient uptake by red blood cells infected with the malaria parasite. Desai SA, Bezrukov SM, Zimmerberg J: *Nature* 2000, **406**:1001-1004.
• **Significance**: The uptake protein for different kinds of solutes into red blood cells (RBC) has been uncertain. Using innovative recording conditions, Desai *et al.* demonstrate that ionic conductances can be recorded in RBCs infected with the malaria parasite. These ion channels are anion selective and may mediate the uptake of solutes. It is unknown whether malaria activates a RBC channel or introduces a parasitic encoded channel protein.
Findings: Whole-cell recordings were obtained by matching oncotic and hydrostatic forces during patch rupture. Whole-cell current revealed inwardly rectifying anion selective current that was inhibited with furosemide (a diuretic) and other Cl^- channel inhibitors. Single-channel recordings showed a 20 pS channel in 1.15 M Cl^-.

AT_1-receptor heterodimers show enhanced G-protein activation and altered receptor sequestration. AbdAlla S, Lother H, Quitterer U: *Nature* 2000, **407**:94-98.
•• **Significance**: Heterodimerisation is emerging as a common theme among G-protein-coupled receptors (GPCRs). What is striking in this paper is that two different GPCRs, angiotensin II (AT_1) and bradykinin (B_2), appear to form heterodimers with enhanced signalling properties and a completely different mechanism of endocytosis.
Findings: Immobilised agonist-bound B_2 receptors were used to identify potential interacting proteins, of which AT_1 was identified. B_2 and AT_1 formed stable dimers in the absence of agonist, with enhanced G protein signalling. Heterodimers internalised via a dynamin-dependent process, in contrast to monomers. B_2 antisense treatment of A10 smooth muscle cells, which express AT_1 and B_2, showed smaller angiotensin-II-dependent changes in intracellular Ca^{2+}.

Cell-to-cell contact and extracellular matrix

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The serpin $\alpha1$-proteinase inhibitor is a critical substrate for gelatinase B/MMP-9 *in vivo*. Liu Z, Zhou X, Shapiro SD, Shipley JM, Twining SS, Diaz LA, Senior RM, Werb Z: *Cell* 2000, **102**:647-655.
• **Significance**: This study uncovers a novel molecular pathway regulating neutrophil-triggered extracellular proteolysis. This pathway might be a key feature in autoimmune diseases, such as bullous pemphigoid (BP).
Findings: Mice deficient in either gelatinase B (GB) or neutrophil elastase (NE) were resistant to skin blistering triggered by injection of antibodies against BP180, a transmembrane component of hemidesmosomes. Using these mice in complemention experiments with neutrophils from wild-type, $GB^{-/-}$ or $NE^{-/-}$ mice showed that GB acts upstream of NE. More precisely, it regulates the activity of NE by inactivating $\alpha1$-proteinase inhibitor, the critical substrate for GB in experimental BP.

The GTPase Rap1 controls functional activation of macrophage integrin $\alpha M\beta2$ by LPS and other inflammatory mediators. Caron E, Self AJ, Hall A: *Curr Biol* 2000, **10**:974-978.
• **Significance**: This study reveals a novel role for the small GTPase Rap1 in $\beta2$ integrin activation of macrophages. Together with two earlier reports indicating such a Rap1 function in T cells (Reedquist *et al. J Cell Biol* 2000, 148:1151-1158 and Katagiri *et al. Mol Cell Biol* 2000, 20:1956-1969), Rap1 emerges as a key regulator of adhesive immune reactions.
Findings: Macrophage phagocytosis induced by phorbol ester, lipopolysaccharide, tumor necrosis factor α or by platelet-activating factor requires activation of the integrin $\alpha M\beta2$. Inhibition or activation of small GTPases in different experimental setups showed that Rap1 specifically controls this process by activating the binding of $\alpha M\beta2$ to complement-opsonized phagocytic targets.

Increased sensitivity to the stimulant effects of morphine conferred by anti-adhesive glycoprotein SPARC in amygdala. Ikemoto M, Takita M, Imamura T, Inoue K: *Nat Med* 2000, **6**:910-915.
• **Significance**: Repeated administration of morphine results in a behavioral response called locomoter sensitization (LS). LS probably results from neuronal hyperactivity in the brain and serves as a model to study biological reactions following chronic abuse of opiate drugs. This report provides solid evidence for a specific brain-associated function of the extracellular matrix protein SPARC (secreted protein acidic and rich in cysteine; also known as BM-40 or osteonectin), which is implicated in LS.
Findings: A subtractive hybridization screen to identify cDNAs induced by chronic administration of morphine in the amygdala of mice revealed SPARC. SPARC mRNA and protein was specifically induced in basolateral amygdala (BA) nuclei by repeated morphine administration and persisted after morphine withdrawal, coincident with the duration of LS. SPARC infusion into BA resulted in LS after only a single injection of morphine.

Cell differentiation

Selected by Peter van Roessel*, Julia Kaltschmidt*, Neil Hukriede† and Michael Tsang†
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Senseless, a Zn finger transcription factor, is necessary and sufficient for sensory organ development in *Drosophila*. Nolo R, Abbott LA, Bellen HJ: *Cell* 2000, **102**:349-362.
• **Significance**: The authors report the characterisation of Senseless (Sens), a novel nuclear protein with proneural-like function.
Findings: *sens* encodes a Zn-finger-containing protein that localises to the nuclei of sensory organ precursor cells. Sens is both necessary and sufficient for sensory organ development. Expression of Sens is dependent on *daughterless* and the proneural gene *atonal*; Sens in turn is required to activate and maintain the expression of further proneural genes.

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***Drosophila* dumbfounded: a myoblast attractant essential for fusion**. Ruiz-Gomez M, Coutts N, Price A, Taylor MV, Bate M: *Cell* 2000, **102**:189-198.
•• **Significance**: In *Drosophila*, differentiated syncytial muscle cells, or myotubes, form by the aggregation and fusion of two classes of myoblasts. *dumbfounded*, described here, is the first gene known to be required for myoblast aggregation.
Findings: *dumbfounded* (*duf*) encodes a transmembrane protein with five immunoglobulin-like repeats. Fusion-competent myoblasts are attracted to endogenous and ectopic sources of *duf* expression. Loss of *duf* expression results in failure of myoblasts to aggregate, thus preventing fusion and formation of myotubes.

Inhibition of adipogenesis by Wnt signaling. Ross SE, Hemati N, Longo KA, Bennett CN, Lucas PC, Erickson RL, MacDougald OA: *Science* 2000, **89**:950-953.
•**Significance**: Wnt signalling is shown to serve as a molecular switch in mesodermal stem cells, mediating the choice between adipocyte and myocyte cell fates.
Findings: Activation of Wnt signalling by expression of Wnt-1 or β-catenin inhibits the terminal differentiation of adipocyte precursor cells. Wnt signalling decreases expression of both C/EBPα and PPARγ, two transcription factors known to promote adipocyte differentiation. The authors show further that inhibition of Wnt signalling in myocyte precursor cells leads to expression of adipocyte markers.

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Distinct β-catenins mediate adhesion and signaling functions in *C. elegans*. Korswagen HC, Herman MA, Clevers HC: *Nature* 2000, **406**:527-532.
• **Significance**: In *C. elegans* three putative β-catenin homologs have been identified: BAR-1, WRM-1 and HMP-2. The authors demonstrate that the signaling and adhesion function of *Drosophila* and vertebrate β-catenin is distributed between separate β-catenin homologs in *C. elegans*.
Findings: BAR-1, WRM-1 and HMP-2 were used in a two-hybrid assay to test for binding to the *C. elegans* Tcf homolog, POP-1 (Tcf transcription factors bind to β-catenin to activate target genes of the Wnt pathway). Only BAR-1 had a direct physical interaction with POP-1. In addition, BAR-1 immunoprecipitated with POP-1 and co-expression of BAR-1 and POP-1 resulted in significant activation of a Tcf reporter. Neither WRM-1 nor HMP-2 were found to coimmunoprecipitate nor coactivate with POP-1. Although BAR-1 was found to be involved in transcriptional activation, it did not function as an adhesion molecule. In two-hybrid assays with the *C. elegans* cadherin homolog, HMR-1, only HMP-2 was found to physically interact. In addition, HMR-1 and HMP-2 coimmunoprecipitate. WRM-1 did not physically interact with HMR-1 or POP-1 and is thought to be part of a divergent Wnt pathway.

Chromatin fine structure profiles for a developmentally regulated gene: reorganization of the lysozyme locus before trans-activator binding and gene expression. Kantaraki J, Hsiu-Hua C, Riggs A, Bonifer C: *Genes Dev* 2000, **14**:2106-2122.
•• **Significance**: The state of chromatin is important for controlling tissue-specific activation and repression. In this study, the authors determine that most chromatin pattern formation is complete before binding of end-stage transactivators, suggesting that heritable chromatin structure is central for guiding development. In addition, using UV photofootprinting, the authors demonstrated, for the first time, that specific nucleotide complexes are already in place in cells that do not have transactivating factors binding to lysozyme cis-regulatory elements.
Findings: The authors examined, at nucleotide resolution level, chromatin fine structure and transcription factor occupancy at the lysozyme early enhancers and at the promoter in lysozyme expressing and non-expressing cells and found the pattern to be similar. Using a novel quantitative UV photofootprinting technique, the authors generated fine-structure profiles of the lysozyme promoter and identified a lipopolysaccharide responsive element.

Mesp2 initiates somite segmentation through the Notch signaling pathway. Takahashi Y, Koizumi K, Takagi A, Kitajima S, Inoue T, Koseki H, Saga Y: *Nat Genet* 2000, **25**:390-396.
• **Significance**: During somitogenesis, each somite is subdivided into anterior and posterior compartments. These compartments differ in both their properties and gene expression. In this study, the authors demonstrate that the Notch signaling pathway plays a role in morphogenesis of both compartments through *mesoderm posterior 2* (*Mesp2*) and *presenilin-1* (*Ps1*).
Findings: Using mouse knockouts, *Mesp2* was found to be important for anterior compartment formation of somites, whereas *Ps1* was found to be important for posterior compartment formation. In addition, *Mesp2* and *Ps1* were found to regulate *Delta-like 1* (*Dll1*) in contrasting manners, in that *Mesp2* is essential for downregulation of *Dll1*, whereas *Ps1* is important for induction *Dll1* expression. Although *Mesp2* and *Ps1* knockouts were found to have opposite effects on *Dll1* expression, both knockouts demonstrated a loss of *Notch1* and *Hes5* expression. To further elucidate the role of *Mesp2* in the Notch signaling pathway, an activated form of *Notch1* was used to replace *Mesp2* (designated $Mesp2^N$). In $Mesp2^N$ homozygous mice, both endogenous *Notch1* and *Hes5* expression was restored.