Cell Cycle and DNA Replication

S05-02

ACTIVATION OF S PHASE PROMOTING CDKs IN YEAST DEFINES A "POINT OF NO RETURN" AFTER WHICH CDC6 CAN NO LONGER PROMOTE DNA REPLICATION

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In eukaryotes DNA replication does not recur until after anaphase. In *Saccharomyces cerevisiae*, assembly of pre-replication complexes (pre-RCs) at future origins as cells exit mitosis (or later during G1) is necessary for initiation triggered by activation of any one of six cyclin B/Cdc28 kinases (Clbs1-6) in late G1. These CDKs remain active throughout S, G2, and M phases, but cells do not re-replicate, presumably due to the absence of pre-RCs during these phases of the cycle. Formation of pre-RCs and their maintenance during G1 depends on synthesis and activity of an unstable protein encoded by *CDC6* . We find that Cdc6 synthesis can only promote DNA replication in a restricted window of the cell cycle: between destruction of Clbs after anaphase and activation of Clb5/ and Clb6/Cdc28 in late G1. The latter corresponds to a "point of no return" after which Cdc6 synthesis can no longer promote DNA replication. In addition, we provide evidence suggesting that Cdc6p associates with Clb/Cdc28 kinases from late G1 until late anaphase. This association might be important for inhibiting pre-RC assembly during S, G2 and M phases. Inhibition of pre-RC assembly by the same kinases that trigger initiation explains how origins are prevented from re-firing until Clb kinases are destroyed after anaphase.

S05-03

CHARACTERISATION OF THE DNA REPLICATION LICENSING SYSTEM IN XENOPUS

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In Xenopus Replication Licensing Factor (RLF) ensures that chromosomal DNA is replicated exactly once in each cell cycle. It consists of two components , RLF-M and RLF-B. RLF-M has been purified to homogeneity and shown to contain at least three members of the MCM/PI family. We will present data to show how the RLF system prevents re-replication of DNA in a single cell cycle. During late mitosis, RLF-B promotes the assembly of RLF-M onto chromatin.

The initiation of DNA replication can only occur on licensed DNA, and as the DNA replicates RLF-M is removed. Rebinding of RLF-M to replicated DNA is prevented by two distinct mechanisms: firstly RLF-B activity is periodic in the cell cycle, being maximally active only in late mitosis; secondly rebinding of RLF-M is dependent upon nuclear envelope permeabilisation or breakdown, suggesting that active RLF-B cannot cross an intact nuclear envelope.

In order to investigate the structure of the licensed 'prereplication complex' we have purified the Origin Recognition Complex (ORC) from Xenopus. XlORC closely resembles the yeast ORC in subunit composition, and immunodepletion experiments demonstrate that XlORC binding to chromosomal DNA is required for the initiation of replication. The results of experiments concerning the interaction of XlORC with the licensing system will be presented.

S05-04

***IN VITRO* DNA REPLICATION IN YEAST NUCLEAR EXTRACTS,** Pasero,P., Braguglia,D., Duncker,B. and Gasser,S.M., Swiss Institute for Experimental Cancer Research, 1066 Epalinges, Switzerland

We have developed an *in vitro* replication system in yeast based on nuclear extracts prepared from cells synchronized in S-phase. This system supports an efficient DNA polymerase α- and δ-dependent replication of plasmid DNA (Braguglia, Pasero & Gasser, *submitted for publication*). However, initiation is not ARS dependent, suggesting that the elements that target the polymerases to origins are missing in this soluble nuclear extract. Intact yeast nuclei were assayed for replication in our soluble extracts. Biotinylated nucleotides were incorporated into nascent genomic DNA and the sites of incorporation were detected by immunofluorescence and confocal microscopy. Replicating nuclei display a characteristic spotted pattern, indicating that replication origins form active centers within yeast nuclei, as it is the case in higher eukaryotes. This DNA synthesis is under a cell cycle control, since G_1 or S-phase nuclei replicate while G_0 or M-phase nuclei do not. Moreover, the initiation of DNA replication is origin specific and ORC and CDC6 dependent. A putative role for the product of the *CDC6* gene in the clustering of replication origins, both *in vivo* and *in vitro*, will be discussed.

S05-05

STRUCTURE AND MODIFICATIONS OF A HIGH MOLECULAR WEIGHT REPLICATION COMPLEX IN MAMMALIAN CELLS

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We have isolated from calf thymus a multiprotein complex active in DNA replication. This complex contains at least DNA polymerase α/primase, DNA polymerase δ and replication factor C. It is functionally active in replication of primed and unprimed single-stranded M13 DNA templates. Replication factor C apparently mediates the interaction of DNA polymerase δ in the complex with proliferating cell nuclear antigen, through an ATP-dependent mechanism. This interaction appears to stabilize the binding of the complex to a template-primer and to coordinate the activity of DNA polymerase α/primase and DNA polymerase δ during replication. Our data suggest the existence of an asymmetric DNA polymerase complex in mammalian cells. Finally, data indicating a possible coordination between the activity of this complex and the cell response to proliferative signals will also be presented.

S05-06

Functional Characterization of *S. cerevisiae* Replication Factor C and Interaction with PCNA and Pol δ

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RFC was originally identified in human cell extracts as one of the cellular factors essential for the replication of SV40 origin-containing DNA *in vitro*. *S. cerevisiae* RFC is a five subunit DNA replication protein with one large subunit of 95 kDa and four small subunits of 36-40 kDa. Biochemical characterization has shown RFC to bind specifically to primer-template structures at the 3'-end of the primer. RFC has an ATPase activity that is stimulated by primed ssDNA and is further increased by interaction with PCNA. Together with PCNA, it facilitates the binding of Pol δ and ε to the primer and increases the processivity of Pol δ.

Analysis of the predicted amino acid sequences revealed similarity among all RFC subunits. They share 7 conserved regions, including a common ATP binding motif and five regions unique to RFC. The large subunits also share a region of similarity with bacterial DNA ligases and, to a lesser extent, poly(ADP-ribose) polymerases. Despite their high similarity, all RFC genes are essential for viability.

We are currently investigating the role of the five ATPase motifs by introducing inactivating point mutations and analyzing the *in vivo* effects by viability and the *in vitro* effects by examining ATPase activity and interaction with PCNA and Pol δ on primed ssDNA.

S05-07

ACTIVATION OF DNA REPLICATION BY CTF/NF-1

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Transcription factors have been proposed to activate origins of replication both in vivo and in vitro. We used an in vivo replication assay based on the metabolic labelling of replicating plasmids, and we have tested whether the transcription activation domain of CTF/NF-1 may activate SV40 origin of replication. GAL4 DNA binding sites were inserted next to the origin and tested for replication in COS cells expressing GAL4 fusion proteins. We found that CTF/NF-1 full length transcriptional activation domain specifically activated replication. Previous data from our and other labs have suggested a role for chromatin remodelling in CTF/NF-1 activity. Point and deletion mutants have been used to address the relevance of the Histone H3-CTF/NF-1 interaction. Indeed we found that the H3 binding domain mediates replication activation. We therefore set up to address the chromatin structure of activated or not activated origins of replication.

S05-08

Induction and individual sensitivity to apoptosis does not depend on cell cycle competence.

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We have developed a rapid assay (24 h) to predict the radiosensitivity of normal tissues based on various white blood-cell types. It is the radiosensitivity of the normal tissues located within the therapeutic beam which poses the primary limitation to radiotherapy success. Six cell-types were analysed: granulocytes, monocytes, B lymphocytes, CD4 and CD8 T-lymphocytes, and natural killer cells. Use of multiple leukocyte cell-types provides an internal control, permits correlation to different therapy-outcome parameters and avoids variation in response resulting from tumour-progression perturbations in the distribution of leukocyte cell-types. Radiosensitivity is indicated as induced apoptosis, scored as the sub-G1 peak on the DNA histogram. Large differences in sensitivity to apoptosis-induction were observed amongst individuals in non-cycling blood cells. The assay is sensitive to low doses of radiation in the centigray region and indications of an inverse dose response have been observed.

S05-09

REPLICATION FACTOR C, AN ESSENTIAL PROTEIN IN DNA REPLICATION AND DNA REPAIR

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Replication Factor C (RF-C) is a heteropentameric protein essential for DNA replication and DNA repair. It is required for loading of proliferating-cell nuclear antigen (PCNA) onto double-stranded DNA and for PCNA-dependent DNA elongation by DNA polymerases δ and ε (Podust, et al, Mol. Cell. Biol., 15,3072-3081,1995). It has the characteristics of a molecular matchmaker. Two cloned domains of the large 140 kDa-subunit turned out to be first the DNA binding domain and second the PCNA binding domain. The PCNA binding domain (aa 478-512) inhibited DNA replication *in vitro* , more specificly by inhibiting the loading of PCNA onto DNA by RF-C and consequently DNA elongation. On the contrary, the DNA binding domain did not show any effect on DNA synthesis (Fotedar, et al., in preparation). By using a modified form of PCNA that can be [^{32}P]-labelled *in vitro* at the C-terminal end, binding of RF-C to PCNA was investigated in kinase protection experiments. The PCNA binding domain of RF-C as well as the heteropentameric RF-C were able to protect PCNA from phosphorylation, suggesting that RF-C binds to the C-terminal sites of PCNA, which are located on one of the sites on the homotrimeric ringshaped molecule.

S05-10

Site directed mutagenesis of human Proliferating Cell Nuclear Antigen (PCNA)

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PCNA is an auxiliary factor necessary for DNA replication and repair. A ring shaped PCNA trimer forms a sliding clamp together with replication factor C (RF-C), tethering pol δ or pol ε to the 3'-OH end of a growing DNA chain. The effect of PCNA trimerization on its interaction with pol δ and RF-C was analyzed by mutating a single tyrosine residue located at the subunit interface (Tyr114) to alanine. This mutation (Y114A) had a profound effect since it abolished trimer formation. Mutant PCNA was unable to stimulate DNA synthesis by pol δ and did not compete with wild type PCNA for pol δ although it was able to oligomerize and could interact with subunits of functionally active PCNA. We conclude that PCNA molecules that are not part of a circular trimeric complex can not interact with the pol δ core. Furthermore the mutant protein could not be loaded onto DNA by RF-C and did not compete with wild type PCNA for loading onto DNA. The adverse effects caused by this single mutation suggest that the trimerization of PCNA is essential for its overall structure as well as for the interaction with other proteins.

S05-11

THE *DMA1* GENE OF *SCHIZOSACCHAROMYCES POMBE* IS A COMPONENT OF THE SPINDLE CHECKPOINT REQUIRED TO ARREST CELLS IN MITOSIS IN RESPONSE TO A DEFECTIVE SPINDLE

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We are interested in studying the mechanism by which the spindle checkpoint operates in the fission yeast *Schizosaccharomyces pombe*. We have cloned years ago the *cdc16* gene and shown that the latter is both required for the coordination of mitosis with cytokinesis, and for the spindle checkpoint function (*cdc16* shares 39% identity with the *BUB2* protein of *S. cerevisiae*). The *dma1* gene (defective in mitotic arrest) was isolated as a multicopy suppressor of the *cdc16-116* mutation. It encodes a 30 kDa protein with a C3H2C3 RING-FINGER motif in the C-terminal region and a FHA domain (Fork-Head associated domain) in the N-term. Deletion of the gene has shown that it is not an essential gene, but cells lacking the *dma1* function are hypersensitive to the microtubule depolymerizing drug MBC and have a higher rate of minichromosome loss than wild type. Experiments in the *nda3KM311* background (cs β-tubulin mutant) have shown that *dma1* is required to maintain high *cdc2* kinase activity and to prevent septation in cells arrested in mitosis. Northern and western blot analysis of synchronized cells have shown that the mRNA and the protein levels are cell-cycle regulated, peaking in mitosis. Ectopic expression of *dma1* gene from an inducible promoter *(nmt1)* in a wild type background leads to a cell cycle arrest. Cells undergo multiple rounds of nuclear division without any septum formation, suggesting that in these conditions *dma1* acts as an inhibitor of septation. Immunofluorescence data together with an analysis of the kinetic of cell cycle arrest have shown that the dma1p inhibits septation by interacting with the actin ring. These results suggest that *dma1* is required to delay mitosis in response to an unfunctional spindle by interacting with the actin ring and preventing septum formation in cells that are still in mitosis.

S05-12

SEQUESTRATION OF REPLICATION FACTORS ON DAMAGED DNA

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Damaged DNA templates pose several problems to DNA polymerases. For example, most modifications of DNA bases disrupt the hydrogen bonding information generating non-instructional (mutagenic) sites. Frequently, DNA synthesis is arrested 3' to or opposite such sites. We have used complementary DNA synthesis in *Xenopus* egg lysates as a model system to study termination of DNA elongation after exposure to UV radiation or the alkylating agent MNU. As expected, DNA synthesis was suppressed by UV photoproducts or base alkylation in a dose-dependent manner, with complete inhibition after exposure of the template to 100 J/m^2 UV light or 1 mM MNU. However, the presence of damaged templates also suppressed complementary DNA synthesis on nondamaged substrates added to the same reactions, indicating that replication proteins (presumably DNA polymerases) become irreversibly bound on damaged DNA. Sequestration of DNA polymerases at potentially mutagenic sites may constitute an early biochemical event in the cytotoxic or apoptotic responses to DNA damaging agents. (Supported by the Wolfermann-Nägeli-Stiftung, Zürich)

S05-13

TELOMERASE AND NEW TELOMERE FORMATION

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The chromatin diminution process in the nematode *Ascaris suum* is a developmentally controlled genomic remodelling that requires the healing of broken somatic chromosomes by the addition of telomeric repeats (see S. Jentsch *et al.*).
To determine if the new telomere formation that takes place at chromosomal breakage sites is mediated by telomerase, we developed an *Ascaris* cell-free system to assay telomerase activity *in vitro*. In these experiments, a telomerase primer is elongated by up to 30 hexameric repeats, a result characteristic of telomeric sequence addition. Because this activity is sensitive to heat inactivation, proteinase K and RNase A digestions, and depends on the time of incubation and concentration of the extract in the reaction, we propose that it can be attributed to a ribonucleoprotein. Altogether, our results strongly argue for the presence of telomerase in embryonic stages where chromatin diminution and chromosome healing occur.

S05-14

BACTERIAL EXPRESSION OF A PEPTIDE ANTIGEN FOR ANALYSIS OF THE RODENT-SPECIFIC p40^{CDK2}

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A p40 variant of the canonical cyclin-dependent kinase 2 (p33^{cdk2}) has been detected in Syrian and Chinese hamster, rat, mouse and guinea pig. The only difference between the two forms is the presence of a 48 amino acid residue long insertion. The cdk2 sequences in rodent species differ slightly, but the insertion appears to be identical. A DNA encoding thioredoxin was introduced into the commercially available pQE30 vector. We subcloned part of the p40 insert into the unique Rsr II site of this pQE30-Trx vector and expressed it as a (6xHis)-thioredoxin fusion protein in *E.coli*. The expression level drastically decreased, but a litre culture still produced a few mg of the target protein which was used for chicken immunization.

S05-15

POLYOMAVIRUS MIDDLE-ANTIGEN-MEDIATED CELL SIGNALING
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Polyomavirus middle-T antigen, the oncogenic early gene product of this virus, transforms cells in culture upon association with various cellular proteins involved in cell signaling, like tyrosine kinases (c-Src, c-Fyn, Yes), phosphatase 2A, phosphatidylinositol 3-kinase (PI 3-K), the adaptor protein Shc and a member of the 14.3.3 family of proteins. In analogy with activated growth factor receptors, middle-T activates the Ras/MAPK cascade upon binding to Shc resulting in constitutive activation and nuclear translocation of MAP kinases in mouse and rat fibroblasts. Using a genetic and a pharmacological approach we found that the synergistic action of PI 3-K was further required for nuclear translocation of MAP kinases by middle-T or serum growth factors. Future experiments will be focused on the identification of the crosspoint(s) between these two pathways. We are also interested in the question whether sustained activation and nuclear translocation of MAPK are sufficient for cell transformation by middle-T.

S05-16

THE STABILITY OF NUCLEOSOMES AT THE REPLICATION FORK

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Purified SV40 minichromosomes were photoreacted with psoralen under various conditions that moderately destabilize nucleosomes. This assay allows indirect distinction between stable nucleosomes, partially unravelled nuclesomes and nucleosomes containing (or lacking) histone H1. In replicating molecules the passage of the replication machinery destabilizes the nucleosomal organization of the chromatin fiber over a distance of 650 to 1100 bp. In front of the fork, an average of two nucleosomes are destabilized presumably by the dissociation of histone H1 and the advancing replication machinery. On daughter strands, the first nucleosome is detected at a distance of about 260 nucleotides from the elongation point. This nucleosome is interpreted to contain no histone H1, while no stepwise association of $(H3\text{-}H4)_2$ tetramers with H2A.H2B dimers on nascent DNA can be detected *in vivo*. The second nucleosome after the replication fork appears to contain already histone H1. The prolonged nuclease sensitivity of newly replicated chromatin described in the literature therefore may not be due to a slow reassociation of histone H1.

S05-17

EFFECTS OF CALMODULIN GENE DISRUPTION IN A CHICKEN B-CELL LINE

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Calmodulin (CaM) is a ubiquitous Ca^{2+}-binding protein which modulates a variety of cellular functions by activating key enzymes. Knock-out experiments of single copy CaM genes in yeast and *Aspergillus* suggested that CaM is essential for cell growth. In vertebrates CaM has been shown to be encoded by at least three genes making genetic experimentation difficult. To unravel the role of CaM in higher eukaryotes we disrupted the CaMII gene in a chicken pre B-cell line (DT40). One allele knock-out cells produce 50% of the wild-type CaMII mRNA whereas in the two alleles knock-out cells no CaMII mRNA is detected. The phenotypes of CaMII(-/-) cells are increased sensitivity towards ionomycin induced apoptosis, a prolonged cell cycle and reduced calcium signal after B-cell stimulation. To confirm these phenotypes we intend to express CaM in knock-out cells in a dosage dependent manner using the tetracycline expression system.

S05-18

MECHANISM OF ASSEMBLY AND ACTION OF EUKARYOTIC REPLICATIVE POL δ (ε) HOLOENZYMES

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The correct duplication of genome and its maintenance in the intact form are crucial to all living organisms. DNA replication and DNA nucleotide excision repair are the complex biochemical reactions involving very sophisticated enzymological machineries. Both these machineries exploit in part the same proteins and include the same events to be executed, namely the assembly and action of the RF-C/PCNA/pol δ (ε) complexes called pol δ or pol ε holoenzymes. These pol δ and pol ε holoenzymes appear to be responsible for the majority of DNA synthesis events on chromosomal DNA. RF-C and PCNA are the key proteins to enable pol δ and ε to replicate (repair) DNA under physiological conditions. They form so called "sliding clamp" which in turn load pol δ (ε) core onto DNA. The place and role of each protein in the action of the whole machinery will be discussed.

S05-19

A SMALL RAS-LIKE GTP-BINDING PROTEIN INVOLVED IN SEPTUM FORMATION IN *SCHIZOSACCHAROMYCES POMBE*

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We have identified a series of genes which play a key role in coordinating mitosis with cytokinesis, in the fission yeast *S.pombe*. Defects in *cdc7*, *cdc11* and *cdc14* genes inhibit septum formation and the cells get highly elongated and multinucleated. *Cdc7* encodes a protein kinase which is a dosage-dependent regulator of septum formation.
Attempts to clone *cdc11* produced only multicopy suppressors so far, but one of them, *sup11X*, shows very strong homology to Ras-like small GTP-binding proteins. *Sup11X* is an essential gene, its disruption causes the cells to elongate and undergo nuclear division without any septum formation. When overproduced, it induces septum formation. Overproduction of *sup11X* in various cytokinesis mutant backgrounds also induces septum formation, with the exception of *cdc7*, suggesting that this gene is downstream of *sup11X*. Moreover overproduction of *cdc7* rescues a null allele of *sup11X*. *Cdc7* could therefore be one of the targets of the *sup11X* protein.

S05-20

VARIATIONS OF CALRETININ EXPRESSION IN THE HUMAN ADENOCARCINOMA CELL LINE HT-29. R. Cargnello, V. Gotzos and M.R. Celio. Institute of Histology and General Embryology, University of Fribourg, Pérolles, CH-1705 Fribourg.

Calretinin (CR) is a Ca^{2+}-binding protein which is not expressed by normal human enterocytes. On the contrary it is expressed by some colon adenocarcinomas. Its function is not yet elucidated, but some evidences lead to hypothesize its involvement in cell proliferation. In order to see whether this protein is related to a particular cellular state, its expression was studied in the adenocarcinoma clonal cell line HT29-18, which can differentiate into enterocytes-like cells by replacing glucose with galactose in the culture medium. After treating the cells with galactose we observed: 1) the appearance of microvilli at the apical surface of the cells, 2) a greater CR expression in undifferentiated HT29-18 cells, grown in glucose-containing medium than in differentiated cells, grown in galactose-containing medium.

Therefore we suggest that CR could be involved in carcinogenesis.

Drosophila and Nematode Embryogenesis

S06-01

Controlling the functional specificity of homeotic proteins

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Much of the *in vivo* functional specificity of the Hox proteins seems to be controlled by poorly understood protein-protein interactions on composite regulatory elements. We have studied such a composite element regulated by the DEFORMED (DFD) protein of *Drosophila*. This *Dfd* epidermal autoregulatory element (*Dfd*-EAE) is also activated in the posterior head of mouse embyros, perhaps in response to mouse DFD-like homologs. Important non-homeodomain binding sites that are required for this element to be regulated by DFD and not other homeotic proteins reside in a 50 bp region that binds a presumptive DFD co-factor protein called DEAF-1.

Other putative homeotic co-factors have been identified in a genetic screen for second-site mutations that enhance weak *Deformed* mutant phenotypes. Using this strategy, we have screened approximately 12,000 third, 9,000 second, and 4,000 X chromosomes for mutations that interact with *Dfd*. Some of the known genes for which mutations were isolated in the *Dfd*-modifier screen are *extradenticle*, *cap n' collar*, and *hedgehog*. How these and other modifier genes contribute to the posterior-head determination pathway along with *Dfd* will be discussed. For example, our results indicate that *extradenticle* is a crucial co-factor for DFD, as it is for many other homeotic proteins. However, its role in controlling the selectivity of homeotic response elements may be mediated through its function as a repressor, not as a selectively binding co-activator with different homeotic proteins.

S06-02

Mechanisms of *wg* signal transduction

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The *Drosophila* segment polarity gene *wingless* and its vertebrate homologue, the mouse *Wnt-1*oncogene, encode secreted growth factor-like molecules that play key roles in generating positional information during development.

In the *Drosophila* embryo, *wg* signaling elicits two distinct cellular responses to pattern the wild-type epidermis. *wg* activity is required for the generation of diverse cell fates in the anterior of each embryonic segment and for the secretion of naked cuticle in the posterior of each segment. These two signaling activities are separately mutable within the Wg protein: EMS-induced mutations have been isolated that disrupt each function independently.

To assess the cellular basis of these altered Wg signaling activities, mutant *wg* transgenes have been constructed and expressed ubiquitously under control of the heat shock promoter. The phenotypic consequences of this manipulated expression will be presented and the implications for *wg* / *Wnt* signal transduction mechanisms will be discussed.

S06-03

Cloning and identification of the *smoothened* gene

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We have identified the transcription unit that rescues the *smoothened* (*smo*) phenotype. The gene encodes a seven-pass membrane protein that has homology to the *Drosophila* Frizzled protein, a putative G-protein-coupled receptor involved in mirror-symmetry pattern formation of adult cuticular structures. The *smo* gene is most abundantly expressed during the blastoderm stage in the trunk region and much less at both poles of the embryo. The gene is also expressed in the head region, similar to the head patch expression of *paired*. At later blastoderm stages, *smo* expression becomes segmentally modulated and remains striped after germ band extension. Since Hh-dependent *wg*-expression is absent whereas Wg-dependent *en*-expression is present in *smo* mutant embryos, the function of *smo* is required in the transduction of the Hedgehog (Hh) and not of the Wingless (Wg) signal. Based on the predicted physical characteristics of the Smo protein and on its position in the Hh signaling pathway, we propose that *smo* may encode the Hh receptor. Its homology to a putative G-protein-coupled receptor may indicate the presence of a G-protein-mediated signal transduction pathway in maintaining positional information within the embryo.

S06-04

***schnurri* is Required for Drosophila dpp Signaling and Encodes a Zinc Finger Protein Similar to the Mammalian Transcription Factor PRDII-BF1**

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Cytokines of the TGFβ superfamiliy were shown to activate receptor complexes consisting of two distantly related serine/threonine kinases. Previous studies indicated that Drosophila dpp (decapentaplegic) which is a homologue of BMP-2 and BMP-4, uses similar complexes and strictly requires the thick veins (type I) and punt (type II) receptor to transduce the signal across the membrane. We present the characterization of the *schnurri* (*shn*) gene and show that it is required for many aspects of dpp signaling. Genetic epistasis experiments indicate that *shn* functions downstream of the dpp signal and its receptors. The *shn* gene encodes a large protein containing zinc finger motifs and is similar to a fimily of vertebrate transcription factors. The shn protein might therefore act as a nuclear target in the dpp signaling pathway directly regulating the expression of dpp-responsive genes.