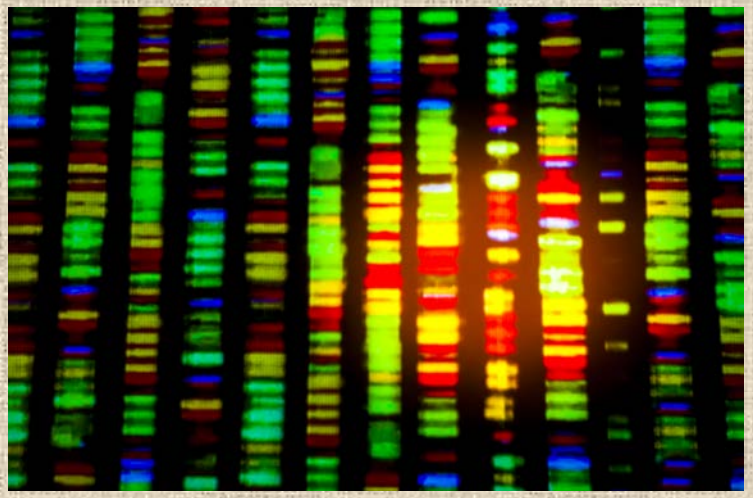
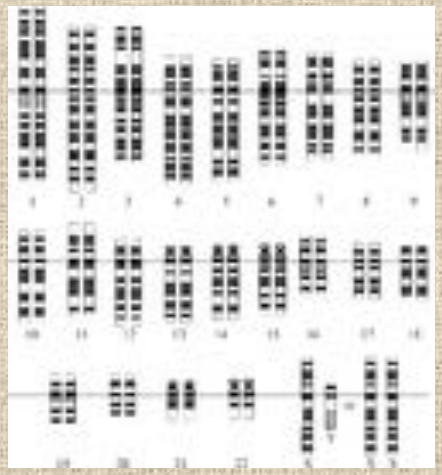
HUMAN GENOME



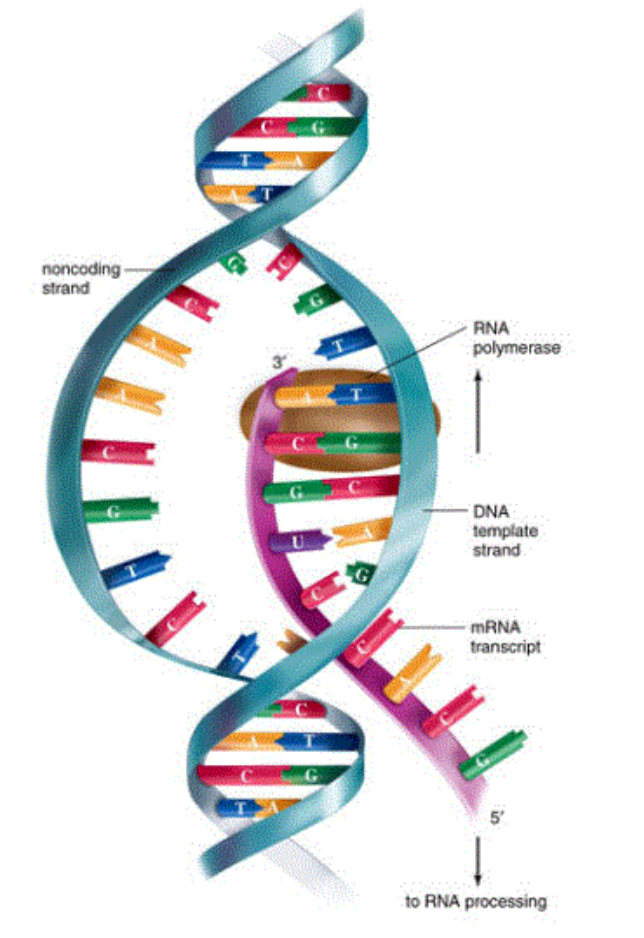
30,000 genes.



23 chromosomes

Length of DNA/chromosome
Six feet

Molecular Aspects of Carcinogenesis
Human Papilloma Viruses
Precancerous Oral Lesions
Papillary\ Lesions

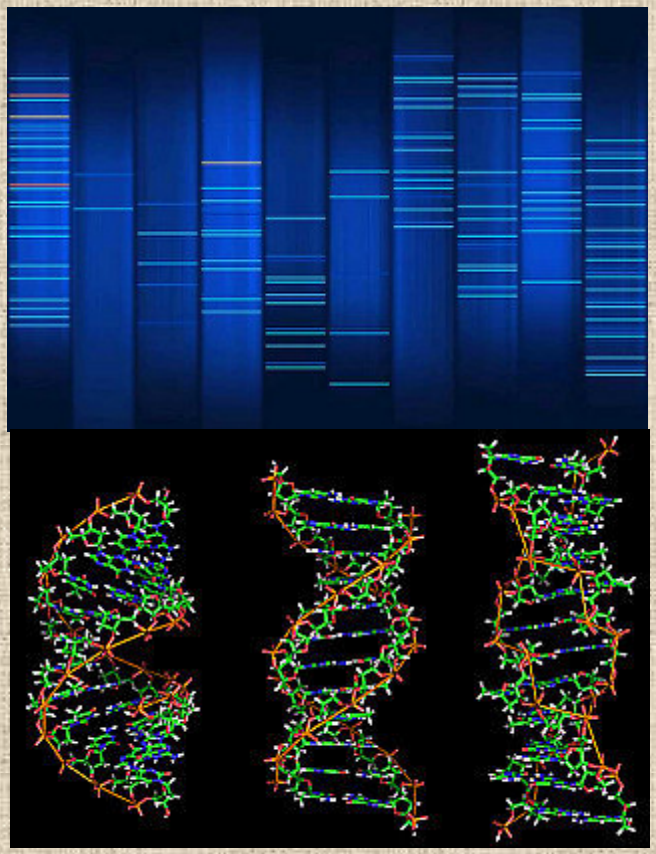


Eversole LR, Leider AS, Jacobsen PL, Shaber EP.
J Am Dent Assoc. 1983 Aug;107(2):248-53.

Oral Kaposi's sarcoma associated with acquired immunodeficiency syndrome among homosexual males.

Abstract
Clinical disease states encountered in the acquired immunodeficiency syndrome (AIDS) have been reviewed with an emphasis on oral Kaposi's sarcoma. The disease is reaching epidemic proportions among homosexual males and is characterized by onset of fever, malaise, diarrhea, and lymphadenopathy. Subsequent to these initial nonspecific signs and symptoms, patients develop a variety of opportunistic infections or Kaposi's sarcoma (or both). The oral lesions of Kaposi's sarcoma are characterized by red, blue, or purple plaques or nodules encountered primarily, yet not exclusively on the palate. Other oral manifestations of AIDS include candidiasis and herpetic stomatitis. Epidemiologic studies suggest the probability of a transmissible agent, perhaps a virus. It is recommended that dental care should be rendered to these patients, using mask and gloves with autoclave sterilization of all instruments.

Cancer Biology



SMOKING GUNS AND FOOTPINTS

MOLECULAR EVIDENCE


LOST
PUPPY

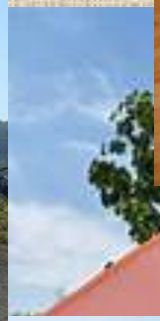
CANCER IS A LESION OF THE GENOME

PROLIFERATION
REPLICATION
ADHESION
VASCULAR PENETRATION
INVASION

ACCELERATION

braking systems

CONSTITUENT PARTS

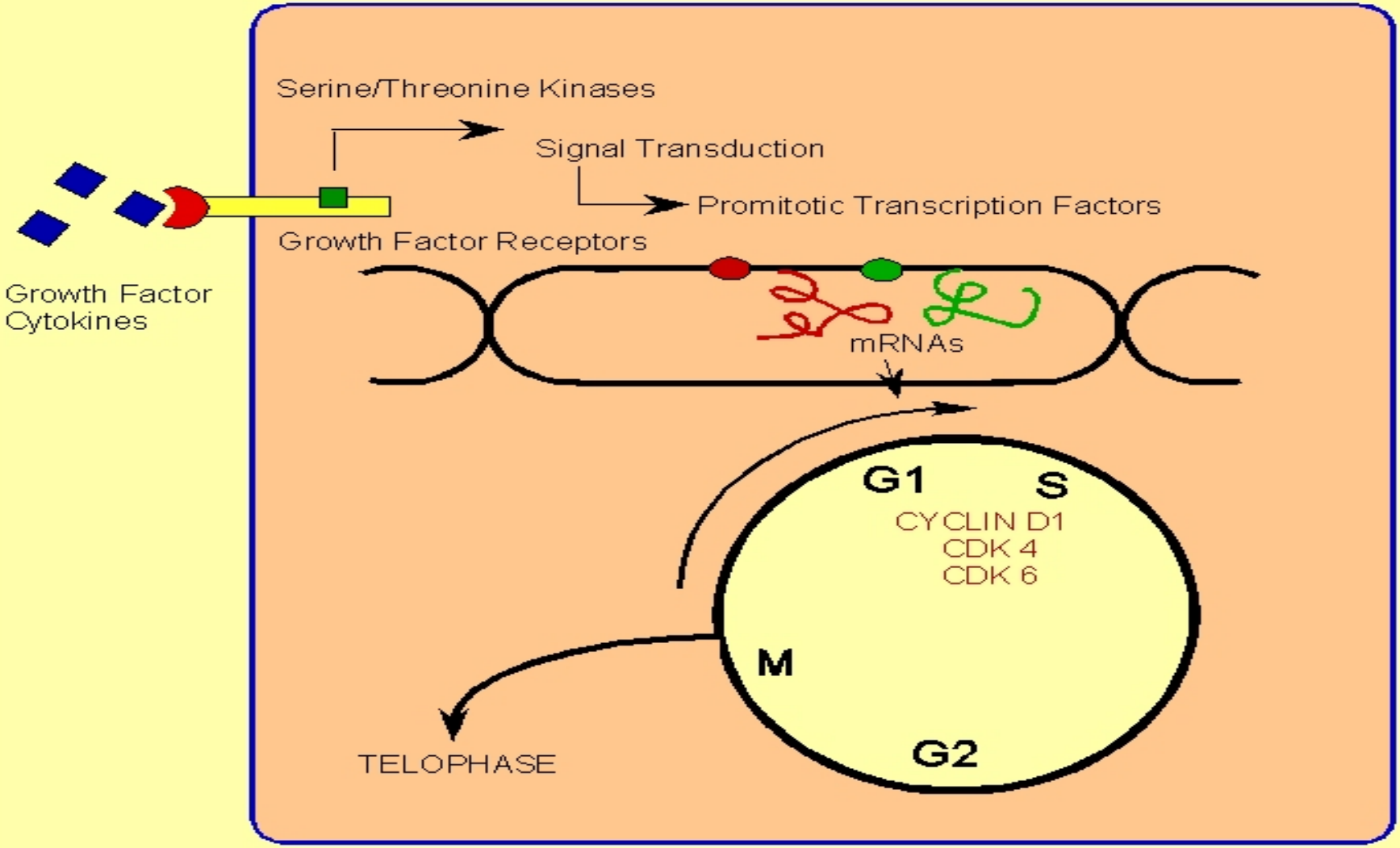


MITOSIS
THE CELL CYCLE

- GROWTH FACTORS, ONCOGENES
- GROWTH FACTOR RECEPTORS
- INTERNAL SIGNALING PATHWAYS
  - PROTEINS AND ENZYMES

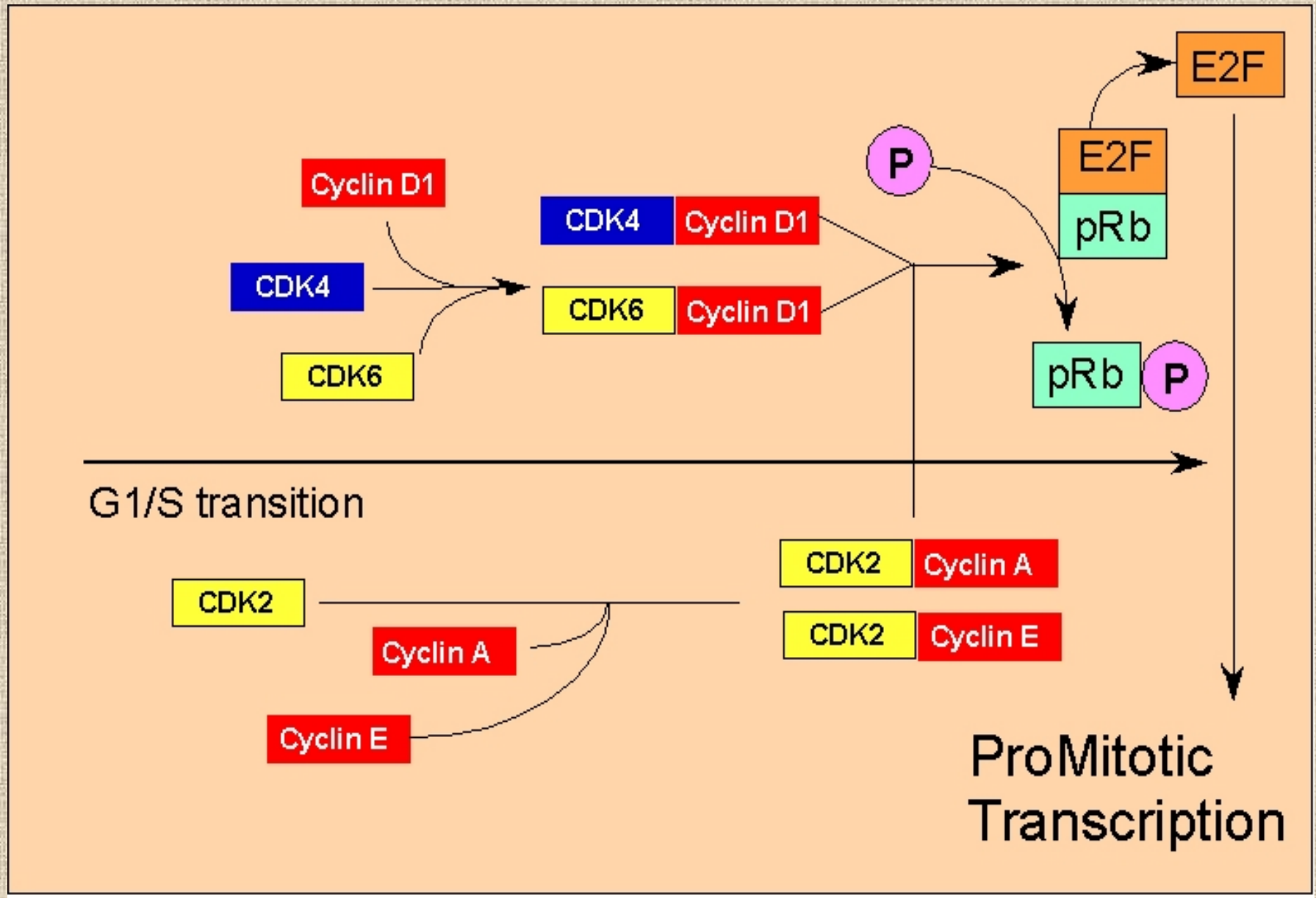
CIRCADIAN RHYTHMS

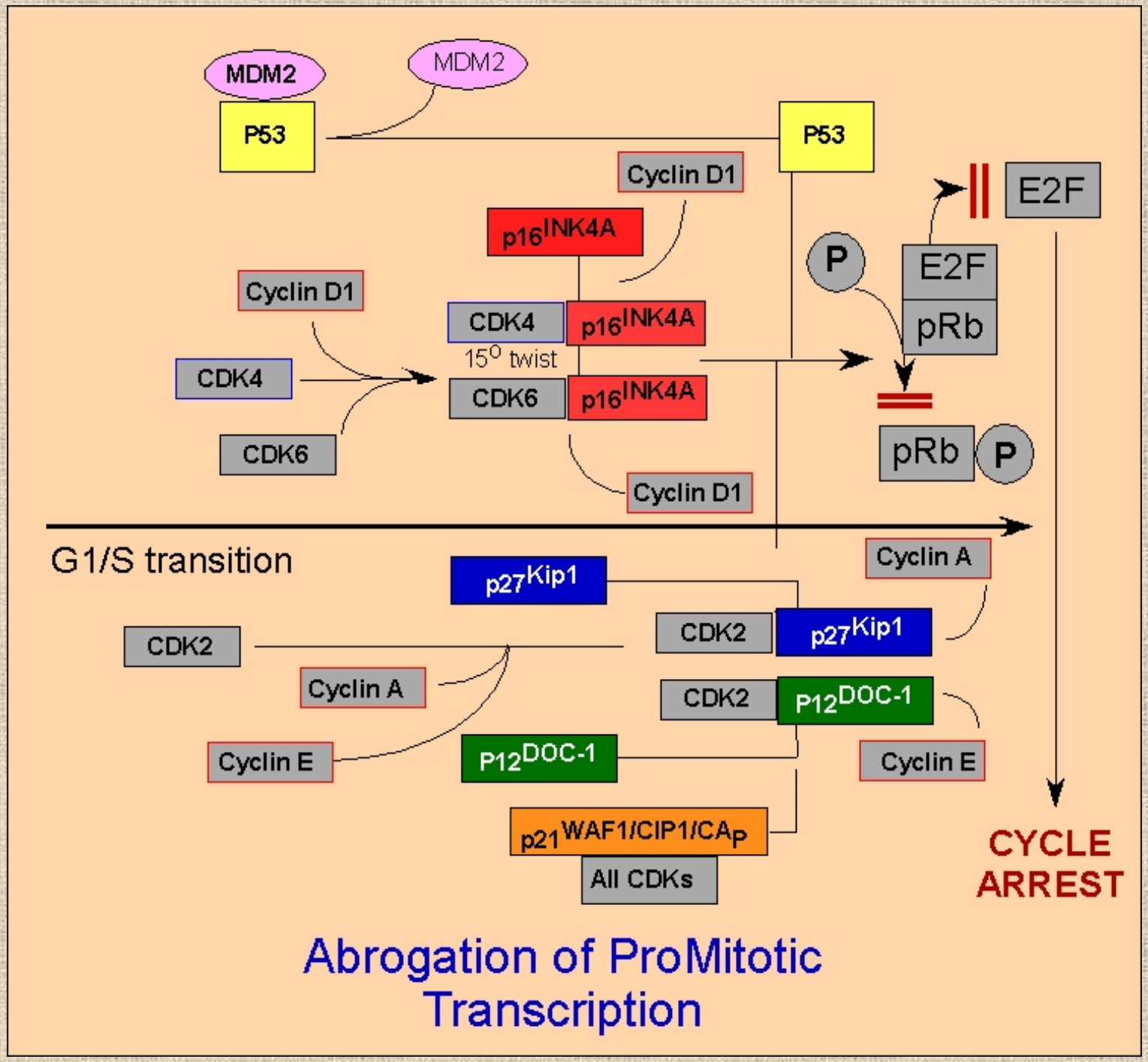
- LOSS OF LEAVES IN FALL, RENEWED IN SPRING
- Menstual cycle
- Tooth eruption
- Cellular clocks


Serine/Threonine Kinases
Signal Transduction
Promitotic Transcription Factors
Growth Factor Receptors
Growth Factor
Cytokines
mRNAs
G1
S
CYCLIN D1
CDK 4
CDK 6
M
G2
TELOPHASE

CELL CYCLE

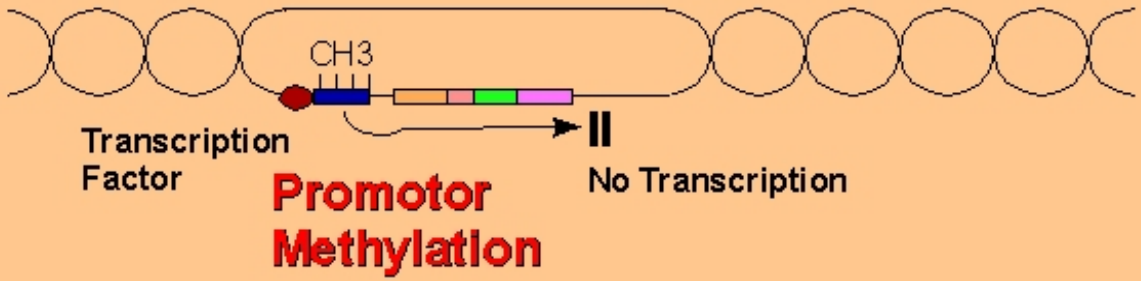
SEVER BRAKE LINE

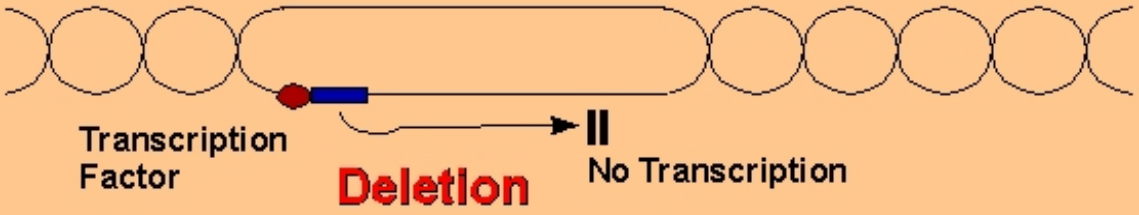

E2F
E2F
pRb
P
Cyclin D1
CDK4
CDK6
CDK4
Cyclin D1
CDK6
Cyclin D1
pRb
P
G1/S transition
CDK2
Cyclin A
Cyclin E
CDK2
Cyclin A
CDK2
Cyclin E
ProMitotic
Transcription

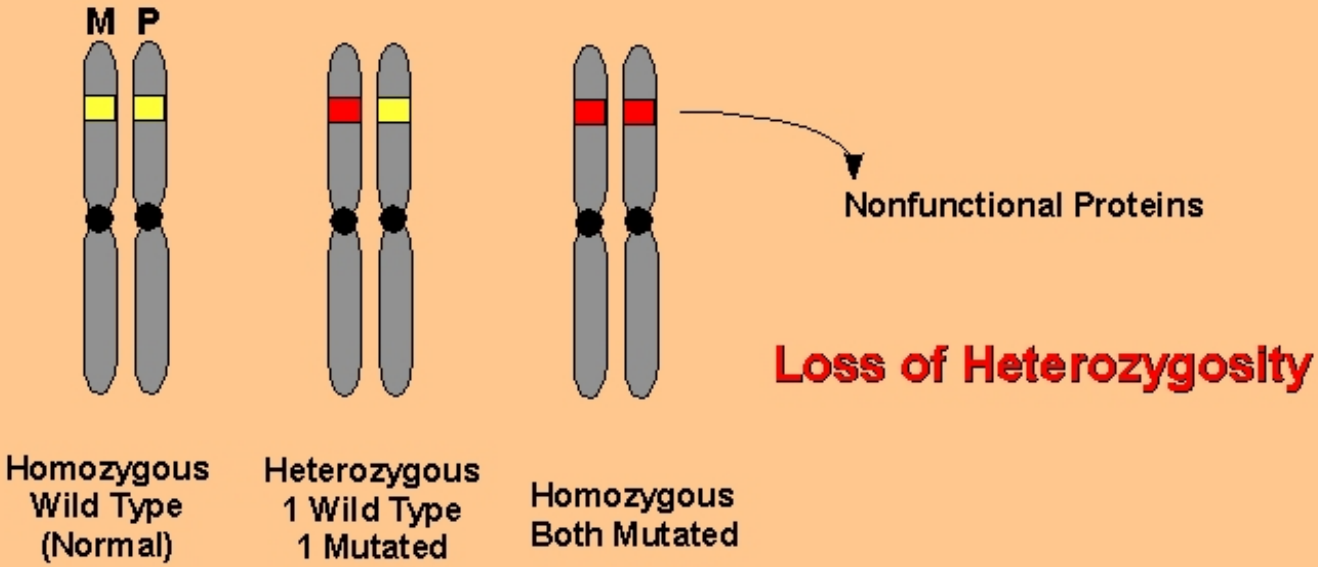


Abrogation of ProMitotic Transcription

Defective Tumor Suppressor Proteins







Tumor Suppressor Proteins in Head and Neck Carcinoma

- LOH or microsatellite instability on various chromosomes and numerous loci: 86%
  - LOH TRAIL Receptor DR4(8p21-22): 47%
  - Methylation or LOH CDKi p16(9p21): 80% early
  - LOH p53(17p13): 50% during invasive stages
  - LOH PTEN: 10%
  - pRb: >10%

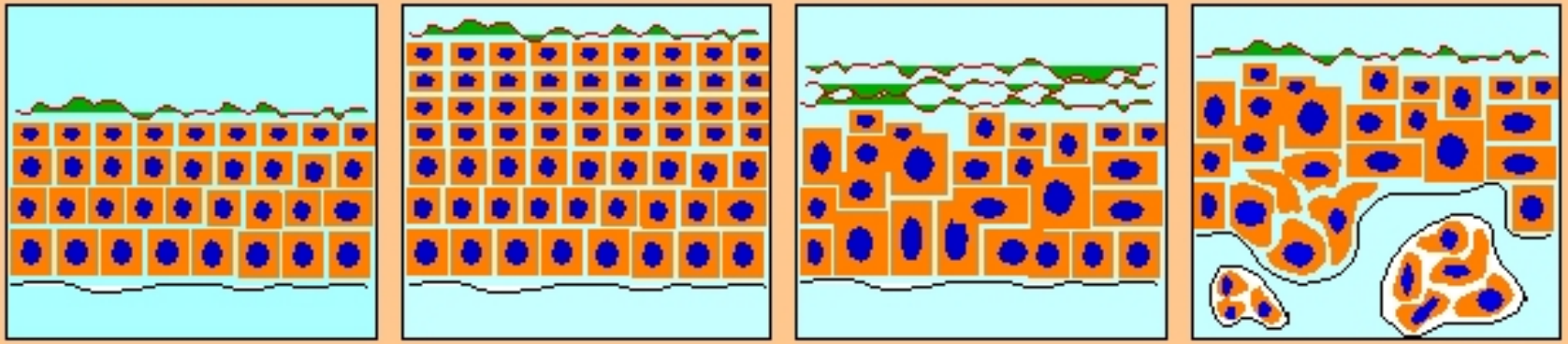
Oral Keratinocyte Carcinogenesis

- The Path to a Malignancy
  - Increased cell cycling
  - Increased keratinocyte motility
  - Keratinocyte-ECM interactions and invasion
  - Tumor cell adhesins that tether to vascular basal lamina proteins
  - Vascular penetration and metastasis
- The Molecular Basis is Multifactoral and Multiphasic with Activation of some and suppression of other genes
  - Mitosis activation
  - Cytoskeletal changes
  - Adhesion molecule expression

Protooncogene Upregulation

- Cyclin D1: 30%
- P63 signaling/migration protein: 30%
- Epidermal GFR: 10%
- C-Myc
- Jun
- RAS

Sequential Molecular Stages in Oral Carcinogenesis



Extant Heterozygous Germ Line Mutations

LOH

HPV

9p12 p16

HPV

17p13 p53

8p21-22 TRAILRec

3p21, 11q13, 13q21, 14q32, 10q23

Oncogene Amp

Cyclin D1 RAS
EGFR MYC JUN

modified from Forastiere, Koch, Trotti and Sidransky: NEJM 345:1890, 2001

Biomarkers

- Biologically expressed phenomena that when detected in a tissue, predict an outcome behavior
- In the conventional sense, a biomarker is demonstrable microscopically by special techniques or may be detectable by molecular or biochemical methods
- In reality, even clinical signs may be considered biomarkers

Histopathological Biomarkers

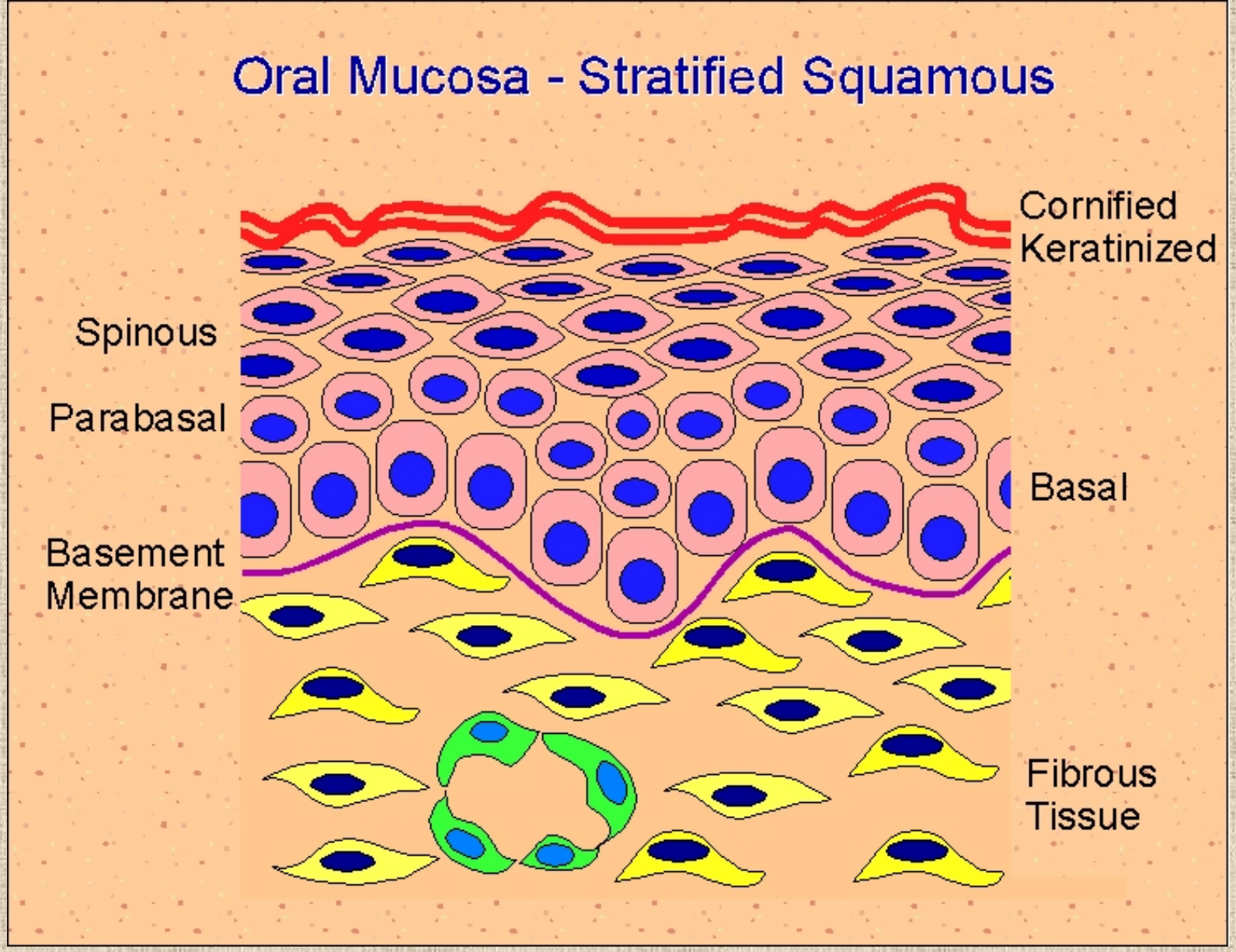
- Expression of Protein, DNA or RNA sequences
- Proteins
  - Immunohistochemistry
- DNA
  - In-situ Hybridization, PCR
- RNA
  - In-situ Hybridization, RT PCR

Biomarker Classes

- Proliferation Markers
  - Cell Cycle Proteins, Oncoproteins, Mutated Tumor Supressors
- Invasion Markers
  - Basal lamina proteins, extracellular matrix, ECM enzymes
- Nuclear Qualitative Markers
  - Computorized Image analyses
- Nuclear Quantitative Markers
  - Ploidy
- Viral Markers
  - HPV 16

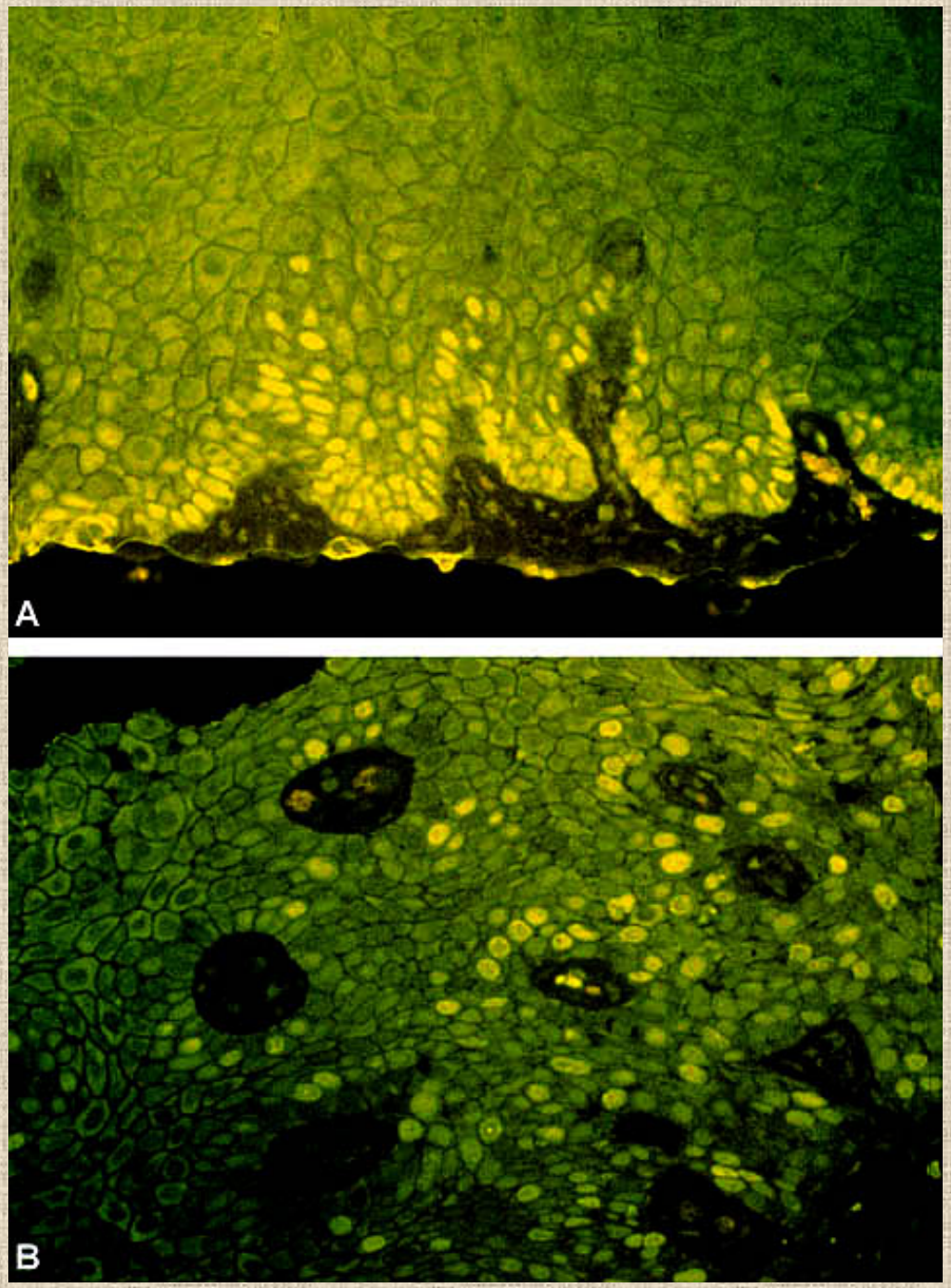
The Ideal Biomarker

- A procedure that can be performed on biopsy specimens
- Expression is easily demonstrable
- Expression of the marker is predictive for cancer progression risk in dysplastic oral lesions
- Expression of the marker is predictive for cancer progression risk in nondysplastic oral lesions
- False negative and positive results do not occur (i.e. expression is 100% predictive for cancer progression, lack of expression is 100% predictive for no cancer progression risk



THE CARDINAL RULE OF ORAL EPITHELIUM: ONLY THE BASILAR AND PARABASILAR CELLS DIVIDE

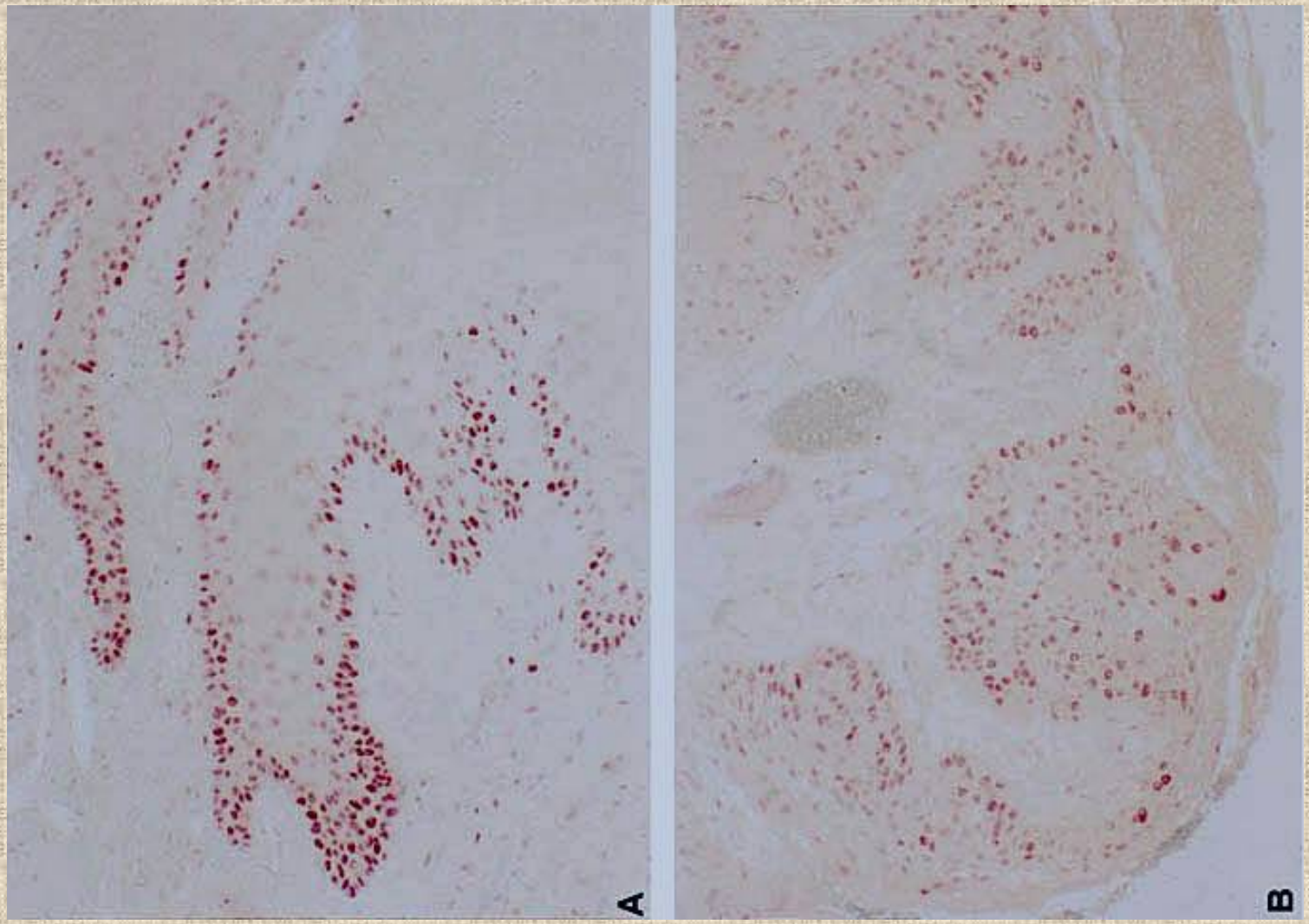
Expression of promitotic genes or their protein products in the spinous cell layer is indicative of intraepithelial neoplasia.



BENIGN KERATOSIS

P53

DYSPLASIA



Benign Keratosis

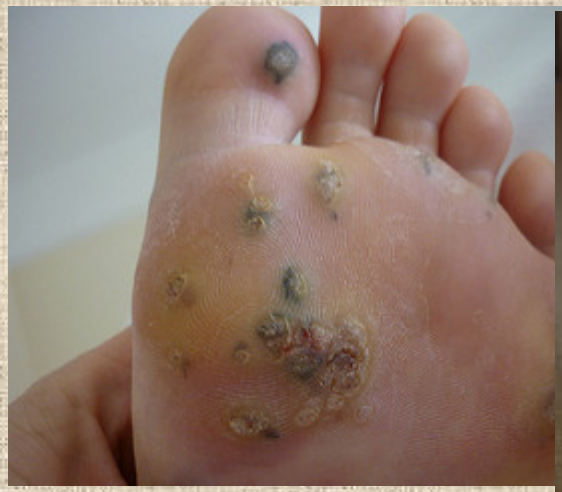
Dysplasia

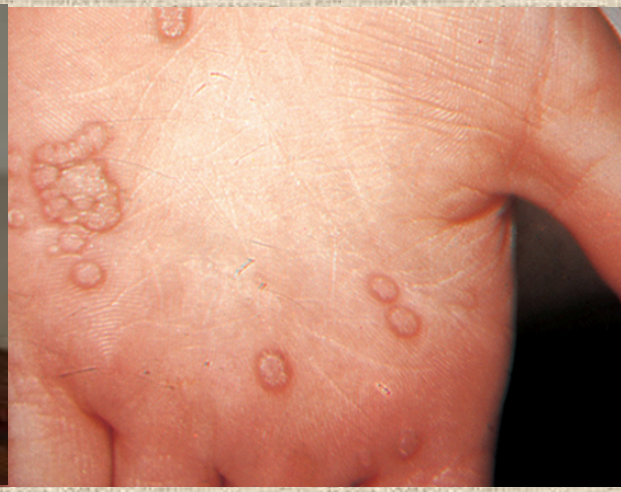
PCNA

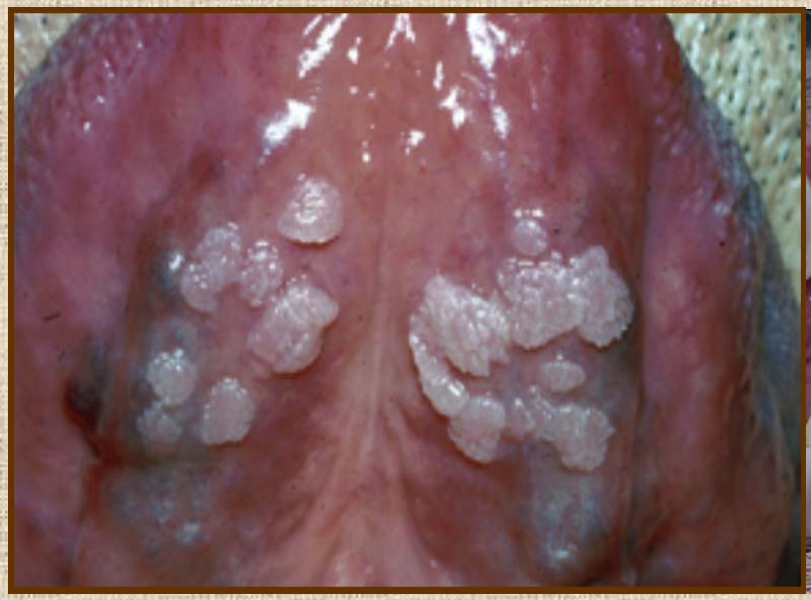
HUMAN PAPILLOMA VIRUS

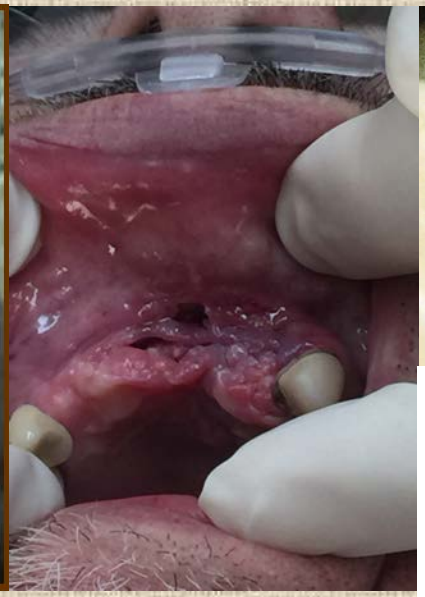
HPV

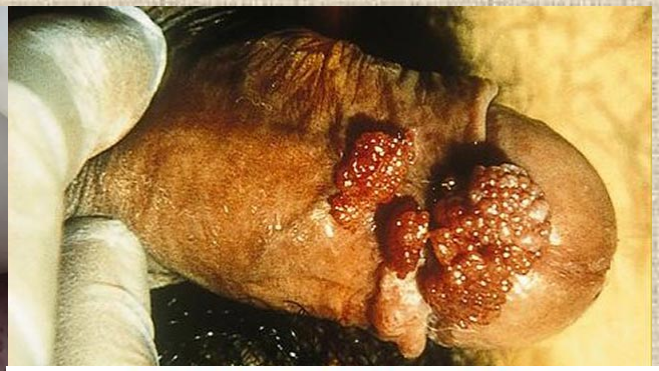
Uterine Cervix

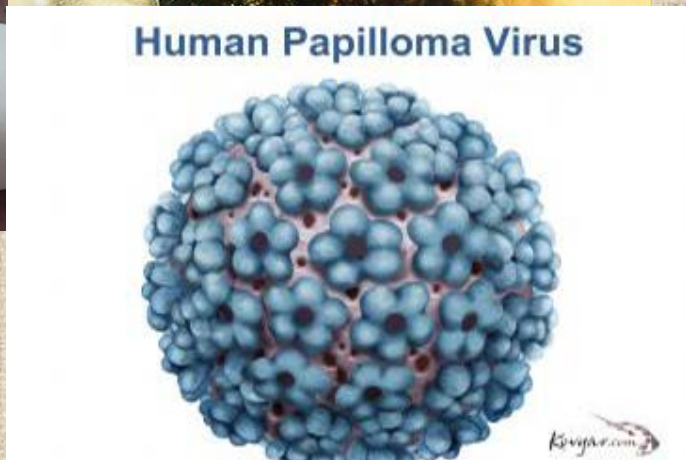











Human Papilloma Virus

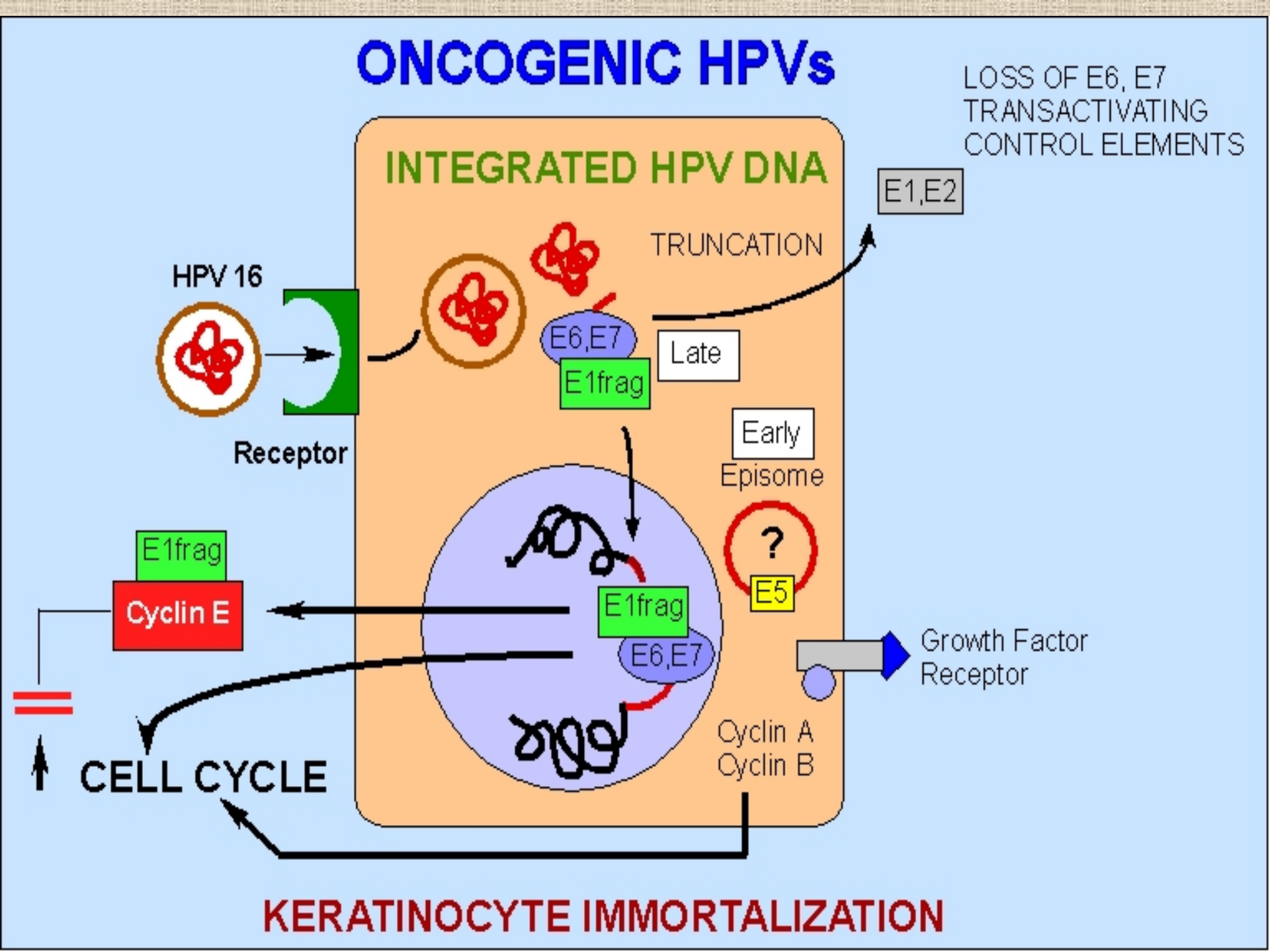
Oncogenic HPVs and Oral SSC

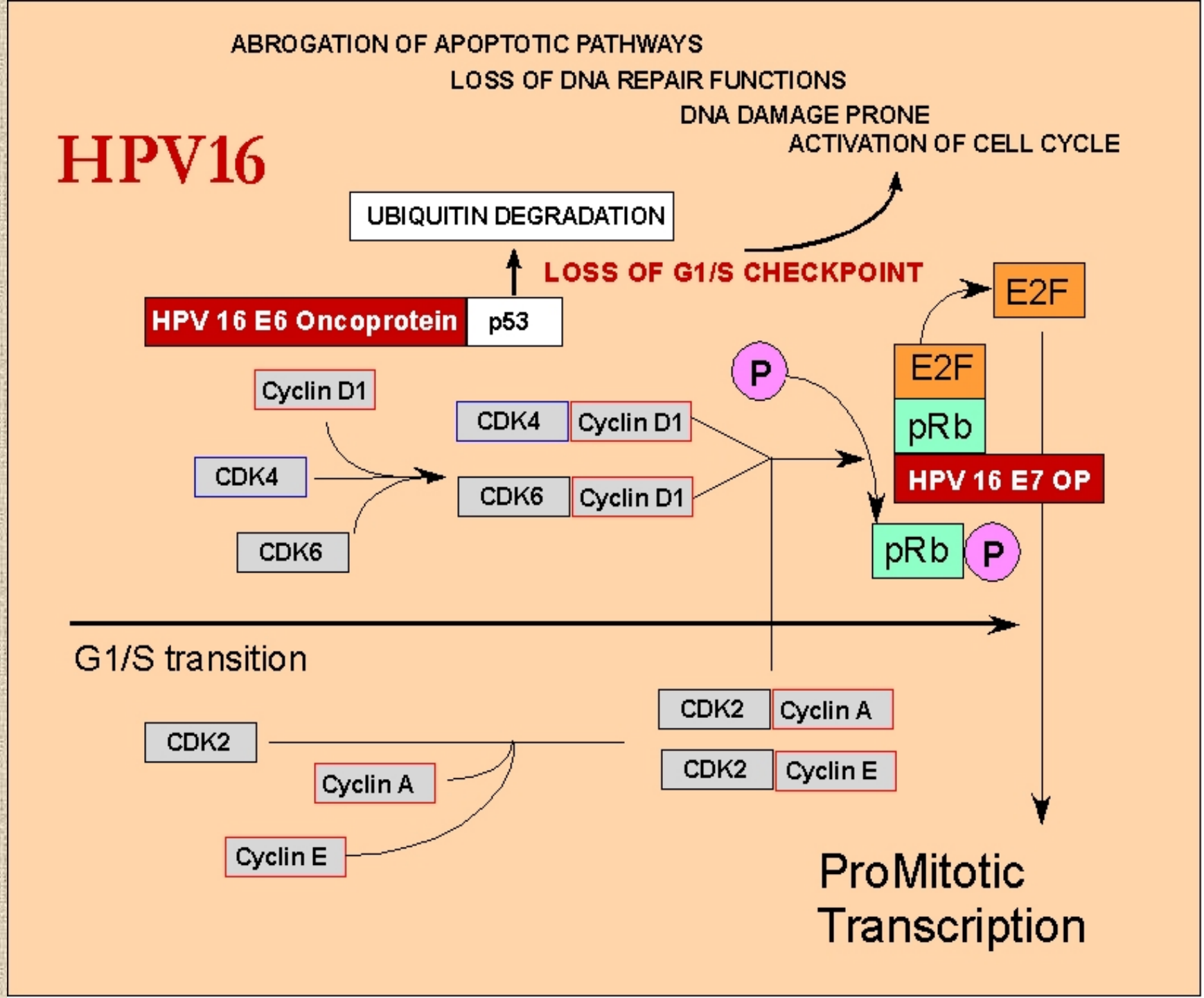
- Oncogenic E6, E7 immortalize oral keratinocytes; Hyperplasiagenic E6, E7 do not
- Oncogenic types are integrated into host DNA and are truncated with loss of the E1, E2 transcription control elements
- HPV is sufficient for Immortalization yet is not sufficient for Tumorigenesis when transplanted into immunodeficient mice
- HPV requires other genetic lesions as cofactors for carcinogenesis
- Infected cells have been shown to express over 80 genes that are not ordinarily expressed in noninfected cells
- The molecular pathogenesis has been shown to be similar to that of CIN and cervical carcinomas

COTTONTAIL RABBIT papillomavirus

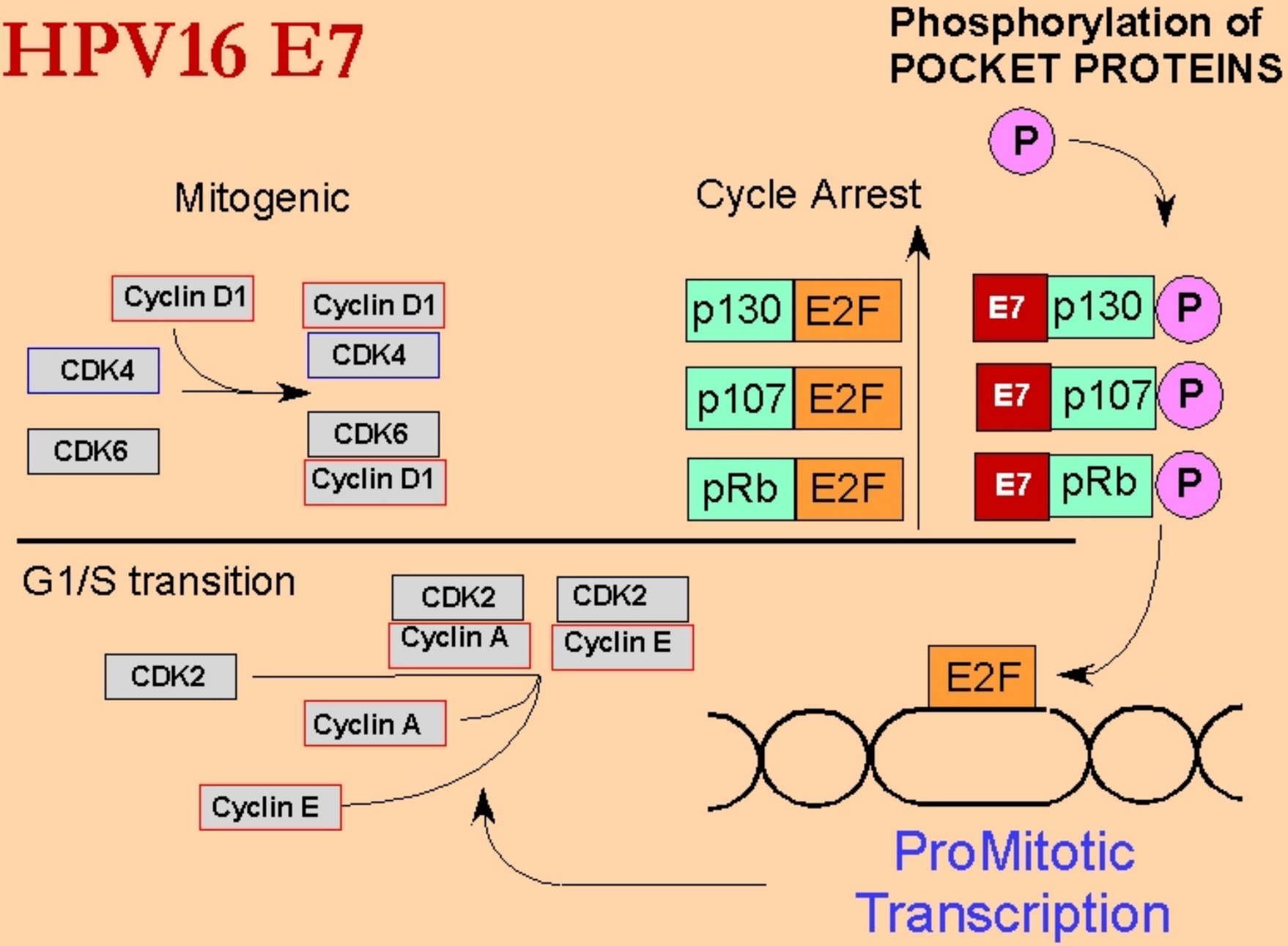
- Papillomas cottontail rabbit

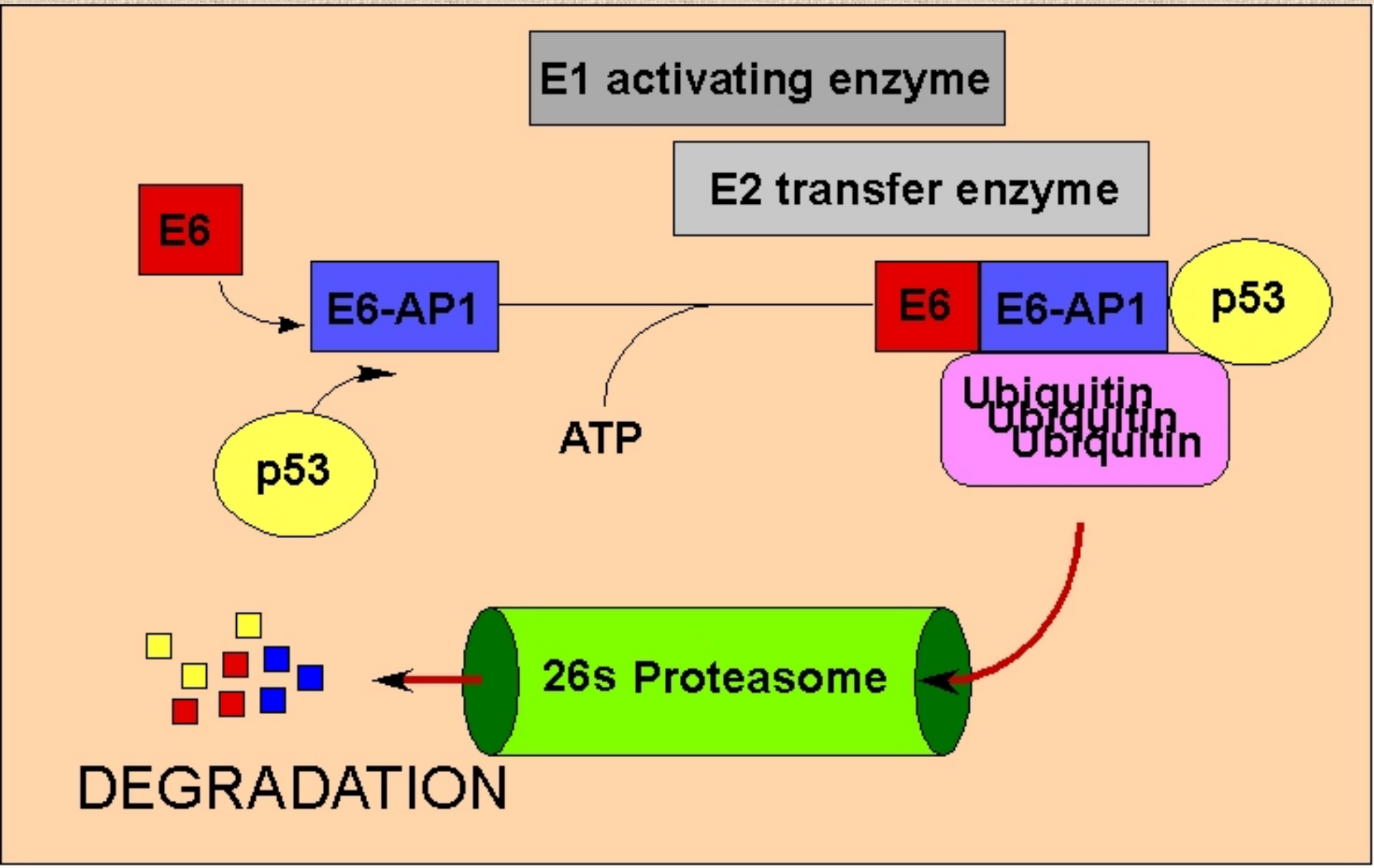
- Human Papilloma Viruses
  - HPV 2, 4 skin and plantar Warts
  - HPV 6, 11 condyloma acuminatum, laryngeal
  - HPV 16, 18 and others carcinoma
    - uterine cervix, vulva, anus, penis, oropharynx, oral cavity


ONCOGENIC HPVs
LOSS OF E6, E7
TRANSACTIVATING
CONTROL ELEMENTS
INTEGRATED HPV DNA
E1,E2
TRUNCATION
HPV 16
E6,E7
E1frag
Late
Receptor
Early
Episome
?
E5
E1frag
Cyclin E
E1frag
E6,E7
Growth Factor
Receptor
Cyclin A
Cyclin B
CELL CYCLE
KERATINOCYTE IMMORTALIZATION


ABROGATION OF APOPTOTIC PATHWAYS
LOSS OF DNA REPAIR FUNCTIONS
DNA DAMAGE PRONE
ACTIVATION OF CELL CYCLE
HPV16
UBIQUITIN DEGRADATION
LOSS OF G1/S CHECKPOINT
HPV 16 E6 Oncoprotein
p53
E2F
P
E2F
pRb
HPV 16 E7 OP
Cyclin D1
CDK4
CDK6
CDK4
Cyclin D1
CDK6
Cyclin D1
pRb
P
G1/S transition
CDK2
Cyclin A
Cyclin E
CDK2
Cyclin A
CDK2
Cyclin E
ProMitotic
Transcription

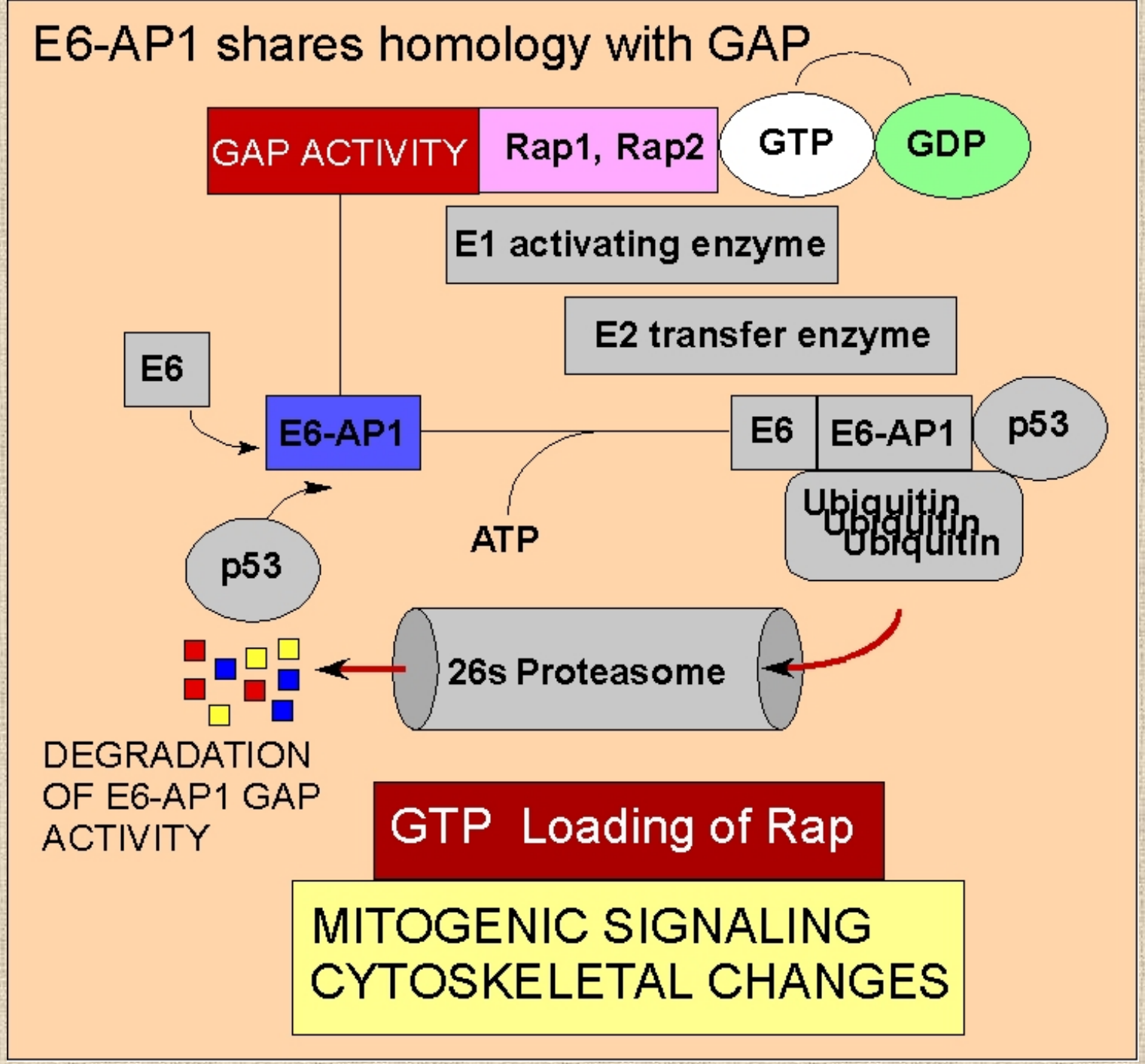
HPV16 E7


Phosphorylation of
POCKET PROTEINS
P
Mitogenic
Cycle Arrest
Cyclin D1
CDK4
CDK6
Cyclin D1
CDK4
CDK6
Cyclin D1
p130
E2F
p107
E2F
pRb
E2F
E7
p130
P
E7
p107
P
E7
pRb
P
G1/S transition
CDK2
Cyclin A
CDK2
Cyclin E
CDK2
Cyclin A
Cyclin E
E2F
ProMitotic
Transcription


E1 activating enzyme
E2 transfer enzyme
E6
E6-AP1
E6
E6-AP1
p53
Ubiquitin
Ubiquitin
Ubiquitin
p53
ATP
26s Proteasome
DEGRADATION

P53 Degradation

- Loss of p53 apoptotic pathway
- G1/S checkpoint over-ride
- Abrogation of DNA repair
- Somatic mutation frequency increased


E6-AP1 shares homology with GAP
GAP ACTIVITY
Rap1, Rap2
GTP
GDP
E1 activating enzyme
E2 transfer enzyme
E6
E6-AP1
E6
E6-AP1
p53
Ubiquitin
Ubiquitin
Ubiquitin
ATP
p53
26s Proteasome
DEGRADATION OF E6-AP1 GAP ACTIVITY
GTP Loading of Rap
MITOGENIC SIGNALING
CYTOSKELETAL CHANGES

HPV E6 has pleiotropic effects!

- Transfection causes immortalization but is not tumorigenic
- E6 binds to p53>ubiquitination
- Binds to Host protein E6-AP, a facilitator for p53 binding; occurs only for oncogenic HPV E6
- Binds to Host protein E6-AP, degradation, activation of Rap1
- Telomere maintainance, activation of telomerase
- Binds to hADA coactivator invoking Retinoic Acid receptor response genes
- Binds hADA coactivator with perturbations in GAP pathways
- Induce mutations independent from p53 checkpoint effects
- Binds DNA 4 way junctions

Is Lichen Planus Precancerous?

- OLP is a relatively common disease, affecting 1 in 200 adults (.5%)(15,000,000)
- 1-2% of OLP patients develop SCCA (1:100, prevalence)
- Carcinoma may develop in the area of a lichenoid lesion or may arise in a nonlesional location
- Oral cancer is a rare disease affecting 35,000 Americans each year (35,000/298,000,000) or 1.2/10,000 (.012%). Incidence

Odds Ratios according to follow up period

83.3 for one year follow-up

8.3 for 10 year follow-up

4.2 for 20 year follow-up

Etiologic Factors in Oral Cancer

- Smoking Tobacco
- Alcohol
- Human Papillomavirus
- Low fruit/vegetable diet
- Lichen planus

HPV VACCINE

GARDASIL 9 is a vaccine indicated in girls and women 9 through 26 years of age for the prevention of the following diseases:

- Cervical, vulvar, vaginal, and anal cancer caused by Human Papillomavirus (HPV) types 16, 18, 31, 33, 45, 52, and 58. (1.1)
- Genital warts (condyloma acuminata) caused by HPV types 6 and 11. (1.1)

And the following precancerous or dysplastic lesions caused by HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58:

- Cervical intraepithelial neoplasia (CIN) grade 2/3 and cervical adenocarcinoma in situ (AIS). (1.1)
- Cervical intraepithelial neoplasia (CIN) grade 1. (1.1)
- Vulvar intraepithelial neoplasia (VIN) grade 2 and grade 3. (1.1)
- Vaginal intraepithelial neoplasia (VaIN) grade 2 and grade 3. (1.1)
- Anal intraepithelial neoplasia (AIN) grades 1, 2, and 3. (1.1)

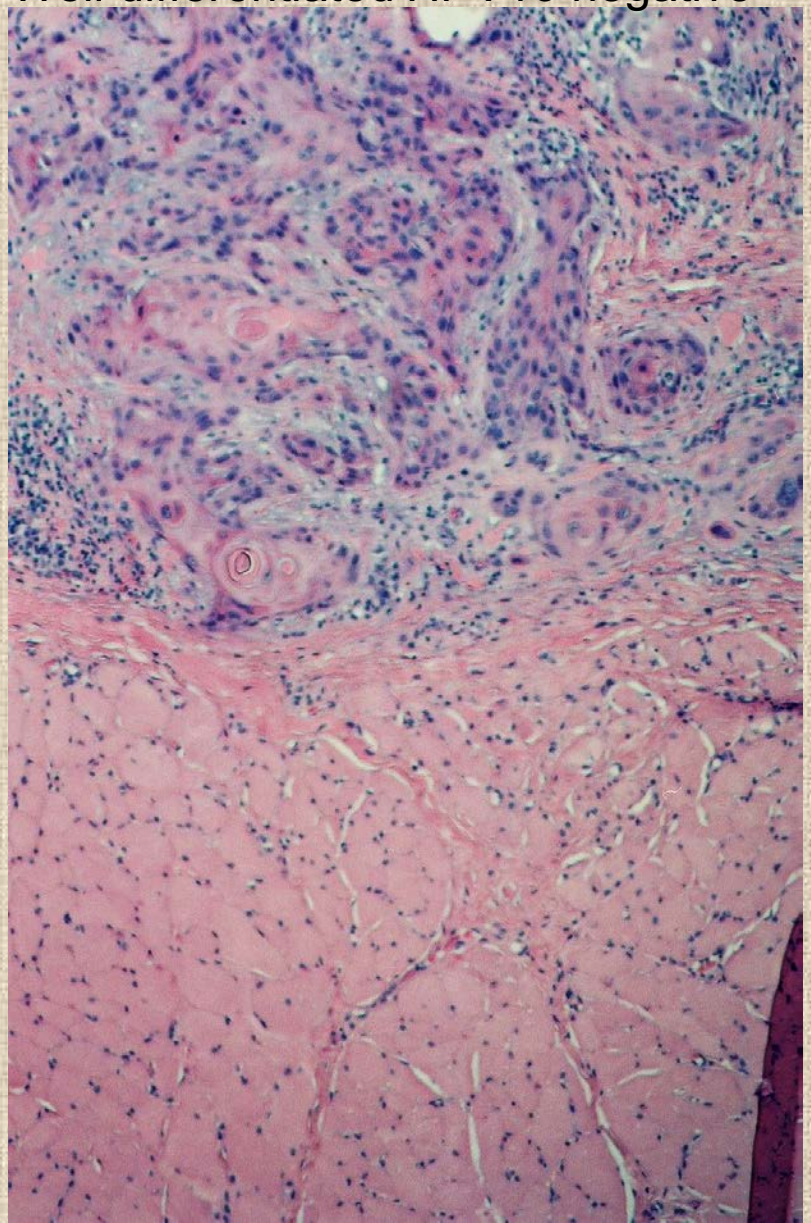
GARDASIL

Age Regimen Schedule 9 through 14 years 2-dose 0, 6 to 12 months* 3-dose 0, 2, 6 months 15 through 26 years 3-dose 0, 2, 6 months *If the second dose is administered earlier than 5 months after the first dose, administer a third dose at least 4 months after the second dose ---------- 0.-mL suspension for injection as a single-dose vial and prefilled syringe.

HPV is an etiologic factor in a subset of Oral SCCAs

- Site: Tongue Base/Tonsillar Region
- Phenotype: Poorly differentiated
- pRb: Phosphorylated inactivated
- P53: Wild Type
- E6, E7: RNA and Protein expressed
- Prognosis: > than nonHPV/p53 CAs
- Metastases: HPV16 DNA present in histopathologically + and – lymph nodes
  - HPV 33, 59 have also been detected in tonsillar CAs

Well differentiated HPV16 negative



Poorly differentiated HPV 16 positive