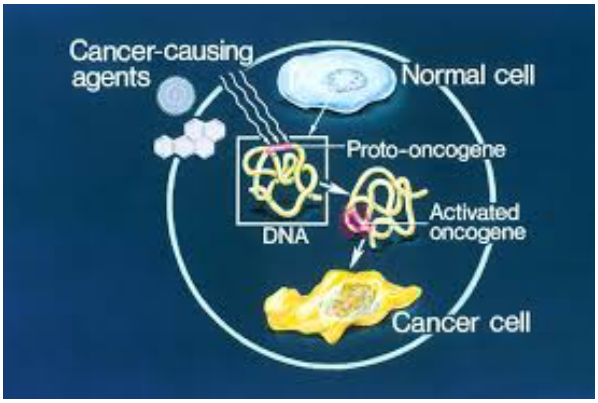
Chapter 3 and 4

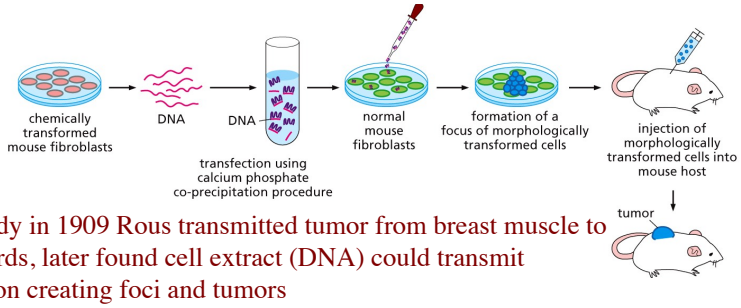
Tumor Viruses and Cellular Oncogenes



Viral sources of Cancer

Viruses were first found in chickens in 1908 by Ellerman and Bang (erythroid leukemia) and in 1911 (soft cell carcinoma) by Peyton Rous.

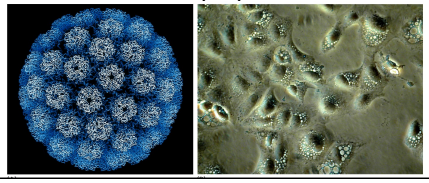
Initial "war on cancer" was thought to be primarily viral

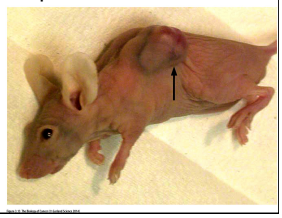


- Initial study in 1909 Rous transmitted tumor from breast muscle to similar birds, later found cell extract (DNA) could transmit information creating foci and tumors
- Showed use the information of cancers was very small (filterable through sand) was a virus.
- At the time (1911) all/most diseases were thought to be infectious in nature – cancer was considered another such disease

Viral sources of Cancer

- The mechanism involves viral DNA being integrated into the host cell DNA, and that the protein products of viral genes maintain transformation to the neoplastic state
- Most viruses are non-transforming - however, they may may play a role in reducing the host cell's ability to inhibit apoptosis.
- Cells that are resistant to apoptosis by virtue of the viral genes that they express are more likely to survive genomic damage that will predispose to later neoplastic damage.
- KSHV (Kaposi sarcoma-related herpes virus has been implicated in AIDS-associated KS, the most common malignant tumor seen in patients with the acquired immunodeficiency syndrome





Viral sources of Cancer

Viral oncogenes (v-onc, i.e. v-Ras) were used to find a large number of transforming cellular oncogenes.

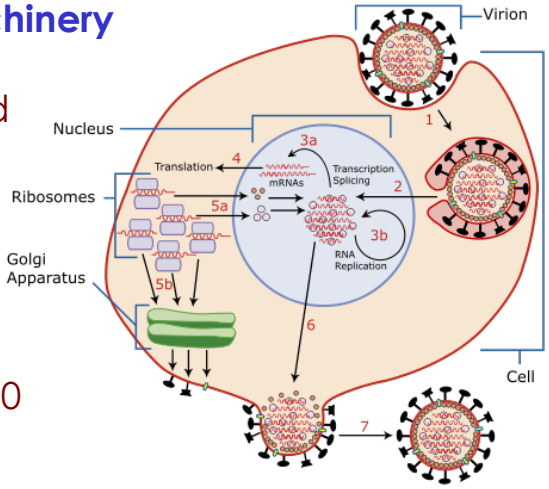
Viral participation in carcinogenesis has turned out to be rare however there are a few well known cases.

- KSHV (Kaposi sarcoma-related herpes virus has been implicated in AIDS-associated KS, the most common malignant tumor seen in patients with the acquired immunodeficiency syndrome.
- Human papilloma viruses are implicated in cervical cancer. There are over 65 varients of the virus and only 10 or so are high risk strains. 85% of the cervical tumors contain the high risk virus. The viral protein seems to interact with pRb or p53.
- Hepatitis B can lead to liver cancer and causes 0.5 million fatalities per year

Life cycle of Virus

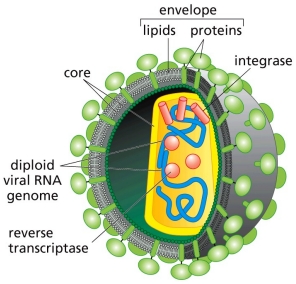
Viron particles bind to proteins on cell surface, shed the coating and replicate using the cells, machinery

- DNA is replicated for new particles
- RNA – Protein for new capsids (protein coating)
- Single cell will produce 100-1000 new virons



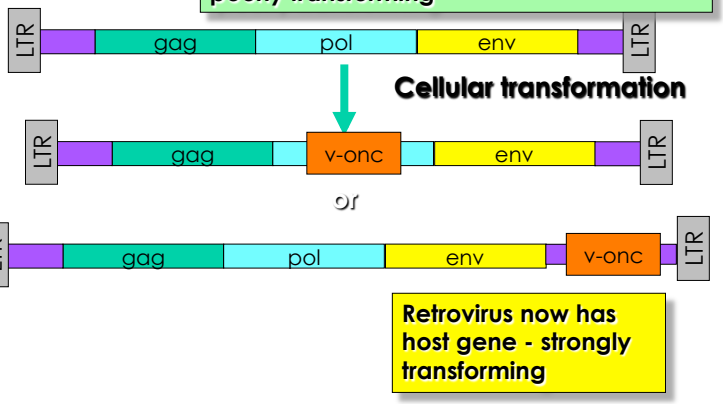
"Virus Replication" by User:YK Times

Retrovirus



HHMI Link (click here)

Retrovirus before it is a viral oncogene - poorly transforming



Retrovirus now has host gene - strongly transforming

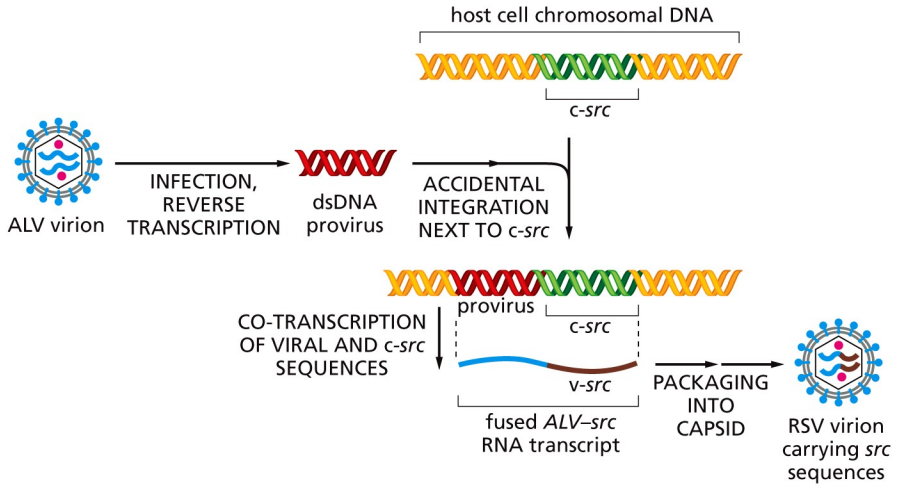


Figure 3.22 The Biology of Cancer (© Garland Science 2014)

Transforming retroviruses and acquired oncogenes

Name of virus	Viral oncogene	Species	Major disease	Nature of oncoprotein
Rous sarcoma	*src*	chicken	sarcoma	non-receptor TK
Y73/Esh sarcoma	*yes*	chicken	sarcoma	non-receptor TK
Fujinami sarcoma	*fps*[b]	chicken	sarcoma	non-receptor TK
UR2	*ros*	chicken	sarcoma	RTK; unknown ligand
Myelocytomatosis 29	*myc*	chicken	myeloid leukemia[c]	transcription factor
Mill Hill virus 2	*mil*[d]	chicken	myeloid leukemia	ser/thr kinase
Avian myeloblastosis E26	*myb*	chicken	myeloid leukemia	transcription factor
Avian myeloblastosis E26	*ets*	chicken	myeloid leukemia	transcription factor
Avian erythroblastosis ES4	*erbA*	chicken	erythroleukemia	thyroid hormone receptor
Avian erythroblastosis ES4	*erbB*	chicken	erythroleukemia	EGF RTK
3611 murine sarcoma	*raf*[e]	mouse	sarcoma	ser/thr kinase
SKV770	*ski*	chicken	endothelioma (?)	transcription factor
Reticuloendotheliosis	*rel*	turkey	immature B-cell lymphoma	transcription factor
Abelson murine leukemia	*abl*	mouse	pre-B-cell lymphoma	non-receptor TK
Moloney murine sarcoma	*mos*	mouse	sarcoma, erythroleukemia	ser/thr kinase
Harvey murine sarcoma	H-*ras*	rat, mouse	sarcoma	small G protein
Kirsten murine sarcoma	K-*ras*	mouse	sarcoma	small G protein
FBJ murine sarcoma	*fos*	mouse	osteosarcoma	transcription factor
Snyder–Theilen feline sarcoma	*fes*[f]	cat	sarcoma	non-receptor TK
McDonough feline sarcoma	*fms*	cat	sarcoma	CSF-1 RTK
Gardner–Rasheed feline sarcoma	*fgr*	cat	sarcoma	non-receptor TK

Example of retroviruse-associated oncognes discovered in altered form in human cancers

Name of virus	Species	Oncogene	Type of oncoprotein	Homologous oncogene found in human tumors
Rous sarcoma	chicken	*src*	non-receptor TK	colon carcinoma[a]
Abelson leukemia	mouse	*abl*	non-receptor TK	CML
Avian erythroblastosis	mouse	*erbB*	receptor TK	gastric, lung, breast[b]
McDonough feline sarcoma	cat	*fms*	receptor TK	AML[c]
H-Z feline	cat	*kit*	receptor TK[d]	gastrointestinal stromal
Murine sarcoma 3611	mouse	*raf*	ser/thr kinase[e]	bladder carcinoma
Simian sarcoma	monkey	*sis*	platelet-derived growth factor (PDGF)	many types[f]
Harvey sarcoma	mouse/rat	H-*ras*[g]	small G protein	bladder carcinoma
Kirsten sarcoma	mouse/rat	K-*ras*[g]	small G protein	many types
Avian erythroblastosis	chicken	*erbA*	nuclear receptor[h]	liver, kidney, pituitary
Avian myeloblastosis E26	chicken	*ets*	transcription factor	leukemia[i]
Avian myelocytoma	chicken	*myc*[j]	transcription factor	many types
Reticuloendotheliosis	turkey	*rel*[k]	transcription factor	lymphoma

Viruses Implicated in Human Cancer Causation

Virus[a]	Virus family	Cells infected	Human malignancy	Transmission route
EBV[b]	Herpesviridae	B cells	Burkitt's lymphoma	saliva
		oropharyngeal epithelial cells	nasopharyngeal carcinoma	saliva
		lymphoid	Hodgkin's disease[c]	
HTLV-I	Retroviridae	T cells	non-Hodgkin's lymphoma	parenteral, venereal[d]
HHV-8[e]	Herpesviridae	endothelial cells	Kaposi's sarcoma, body cavity lymphoma	venereal, vertical[d]
HBV	Hepadnaviridae	hepatocytes	hepatocellular carcinoma	parenteral, venereal
HCV	Flaviviridae	hepatocytes	hepatocellular carcinoma	parenteral
HPV	Papillomaviridae	cervical epithelial	cervical carcinoma	venereal
JCV[f]	Polyomaviridae	central nervous system	astrocytoma, glioblastoma	?

Behaviors of transformed cells

Monolayer cells stop growing upon touching each other (contact inhibition)

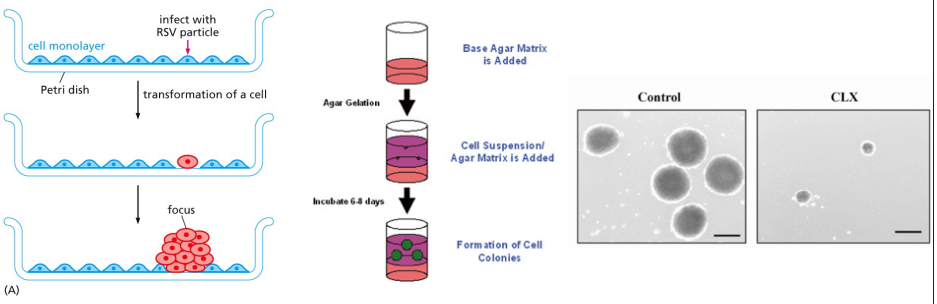
- transformed cells lose this phenomena

- they also lose density inhibition and can form clusters of cells (foci) displaying a loss of topoinhibition.

Transformed cells excessively proliferate for long (indefinite?) passages – immortalization

Non-transformed monolayer cells (epithelial…) grow poorly without solid substrate – anchorage dependence - can grow suspended in agar

Increased Tumorigenicity and transport of glucose



Protoncogenes / Oncogenes

Virus/Retrovirus use proto-oncogenes as a strategy for survival and propagation, but most cancers are not viral in nature

- Proto-oncogene: a normal cellular gene that upon alteration of DNA can acquire the ability to function as an oncogene
- Oncogene: a protein capable of inducing cancer (can transform cells).
  - c-ZYZ indicates a cellular, yet to be oncogenic (mutated) proto oncogene of cellular origin vs
  - v-XYZ is a viral oncogene origin (not often used)
  - Non-human oncogenes are in italics *Xyz*
  - Oncoproteins (not genes) start with capitals followed by lower case Xyz vs XYZ

Protoncogenes / Oncogenes

Protein (molecular) basis of cancer is found at the genetic level

- Malignant transformation occurs by chromosomal damage, proto oncogene mutation or increase in DNA activity
- DNA is the critical macromolecule in cancer. Daughter cells will retain the mutations and the transformation phenotypes and continue to recruit normal cells into transformation

Genetic Basis of Cancer

-Viral oncogenes - insert mutated DNA into cell and create oncogenes

-Translocation of chromosomes - movement of one segment of a chromosome to another

– not normally a cause of cancer but used to find cellular proto oncogenes and study their effects

-Point Mutations - Alterations in specific sequences of critical genes (proto oncogene activation)

- usually needs several mutations with one or more critical requirements for cancer to develop

-Alteration in promoter/enhancers - can occur due to chromosomal translocation (expression)

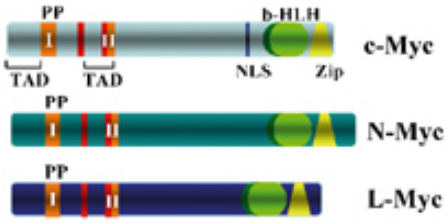
-Gene amplification (expression)

Retroviral – Gene Amplification

Increased copy number of a gene can lead to transformation, proliferation and cell migration or enhanced survival.

- **Lead to increased protein production and as a result higher signaling**

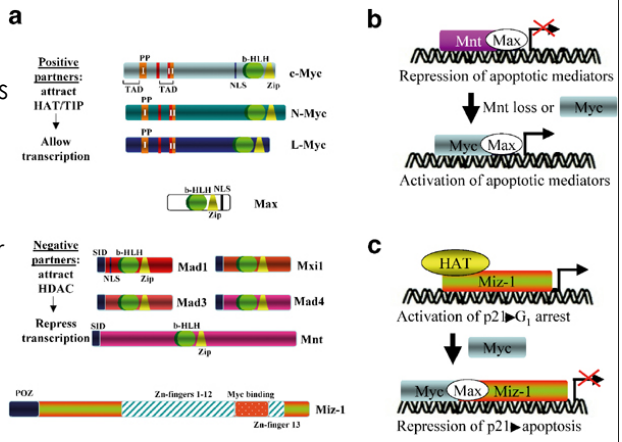
Myc – first found via avian MC29 **my**elo**c**ytomatosis transforming virus is 10-20 extra copies in leukimia and many other cancers



Retroviral – Gene Amplification

Myc –cellular (c-Myc) homolog and isoforms N, L and other Myc forms expresed and activated in nearly 70% of cancers.

- Is a transcription factor (helix-loop-helix leucine zipper) found in most organisms.
- Leads to activation via binding partners to activate proliferation and several signaling cascades – promising target for therapy as small inhibition seems to reduce tumor formation



Gene Amplification

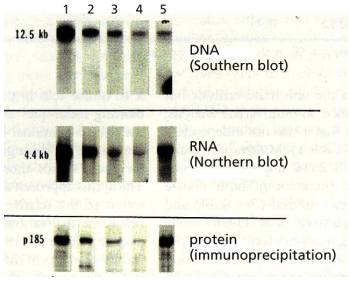
erbB – first discovered in avian erythroblastosis virus – increased copy number in stomach, breast and brain tumors and many others.

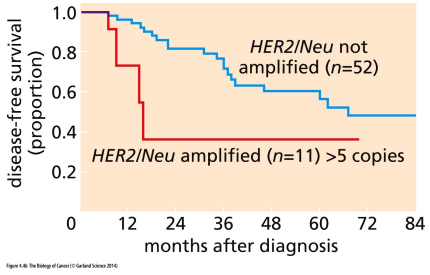
- aka – Neu/Her/erbB: EGFR over expression – separate from mutation/ truncation. Several subforms generate protein tyrosine kinase receptors.
  - Gene is amplified in 30% of breast cancers and are the target of several antibody drugs (biologics) Herceptin.

Gene Amplificaton

Direct correlation of amplification with poor breast cancer prognosis

- > 5 copies increases tumor formation and decreases 5 year survival rate by nearly half.
- Gene amplification alone can't explain breast cancer
  - DNA amplification increases protein but mRNA can be increased independently.
  - Some amplifications stretch beyond Myc to enhance other oncogenes providing a synergistic effect





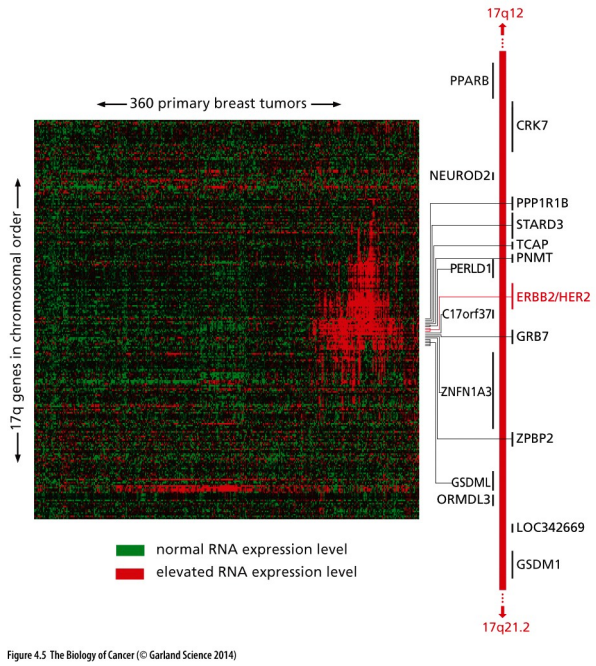


Figure 4.5 The Biology of Cancer (© Garland Science 2014)

Gene mutations – may cause protein structural changes in oncogene activation

Point mutation – single DNA base pair can lead to altered amino acid and protein function. **Ras is an example of a somatic mutation leading to cancer**

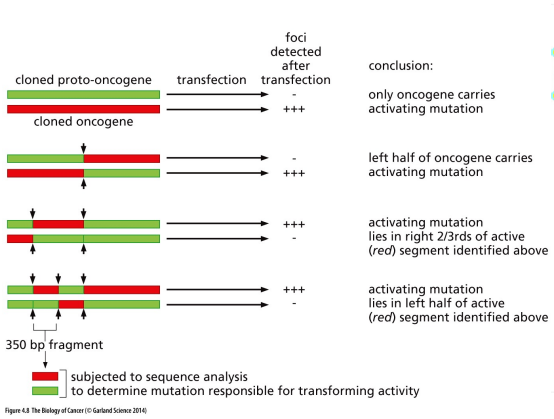
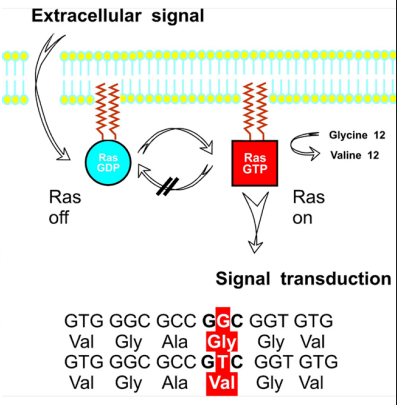
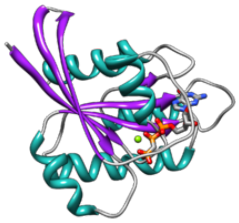


Figure 4.8 The Biology of Cancer (© Garland Science 2014)



Point Mutation - Ras

Rat Sarcoma discovered by Jennifer Harvey and Werner Kirsten



Three human Ras isoforms (3 different genes)

- NRas – initially found in neuroblastomas
- Kras – Kirsten – sarcomas
- Hras – Harvey rat sarcomas.

P-loop

- Binds second phosphate of GTP
- Gly-Val mutation decreases GTPase activity leaving Ras active

Tumor type	Proportion (%) of tumors carrying a point-mutated *ras* gene[a]
Pancreas	90 (K)
Thyroid (papillary)	60 (H, K, N)
Thyroid (follicular)	55 (H, K, N)
Colorectal	45 (K)
Seminoma	45 (K, N)
Myelodysplasia	40 (N, K)
Lung (non-small-cell)	35 (K)
Acute myelogenous leukemia	30 (N)
Liver	30 (N)
Melanoma	15 (N)
Bladder	10 (H, K)
Kidney	10 (H)

[a]H, K, and N refer to the human *H-RAS*, *K-RAS*, and *N-RAS* genes, respectively.

Enhanced gene – protein activity via translocation

Placement of oncogene in front of active gene/chromosomal arm will increase protein expression without gene copy

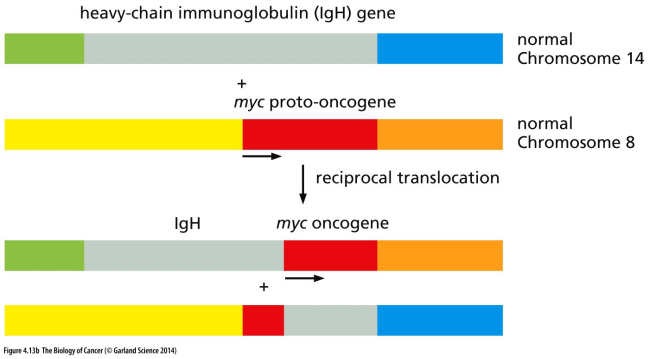


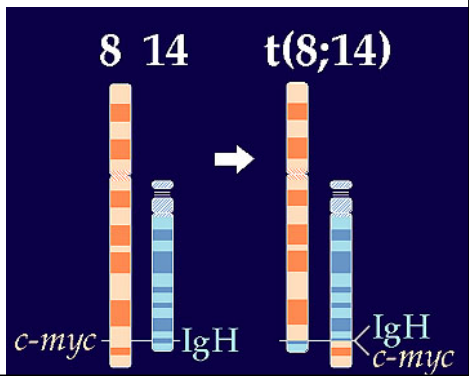
Figure 4.13b The Biology of Cancer (© Garland Science 2014)

Chromosomal Translocation:

Translocation between chromosomes 8 and 14 found in Burkitt's lymphoma (lymph system cancer / leukemia)

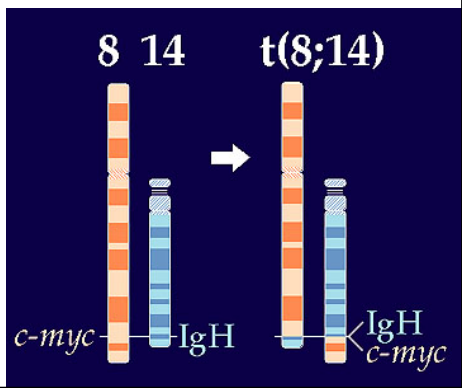
Burkitt's lymphoma is a B cell neoplasm characterized by small noncleaved cells that are uniform in appearance. This neoplasm is one of the fastest growing malignancies in humans.

Burkitts lymphoma is characterized by a specific cytogenetic defect, a balanced, reciprocal translocation of genetic material from the long arm of chromosome 8 to the long arm of chromosome 14.



Chromosomal Translocation:

lymphoma are characterized by a specific cytogenetic defect, a balanced, reciprocal translocation of genetic material from the long arm of chromosome 8 to the long arm of chromosome 14. Two variants of Burkitt's lymphoma are recognized: African and non- African; although very similar in histologic and cytologic features, they have very different epidemiologic patterns and clinical presentations. African Burkitt's lymphoma presents most often as a jaw or orbital tumor and occurs endemically in central Africa. In contrast non- African Burkitt's lymphoma presents primarily as an abdominal mass.

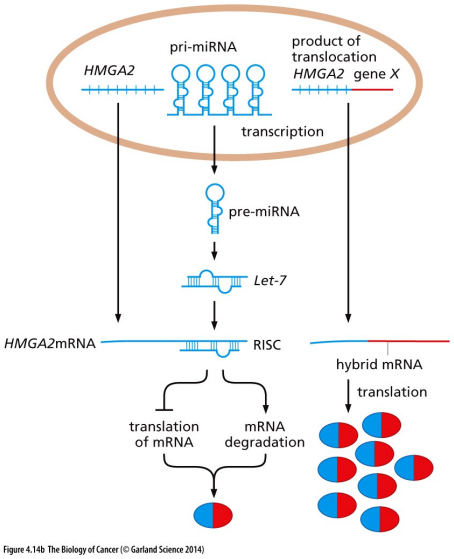


Translocation – micro RNA

Nuclear Protein HMGA – translocates in a non-protein coding region to another gene producing a hybrid protein.

Micro-RNA silencing (shRNA/RNAi) DNA is left behind.

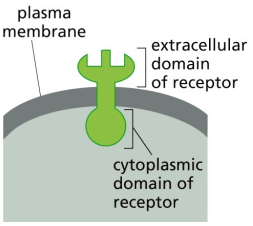
Loss of degredation of mRNA for HMGA leads to enhanced and extended protein levels

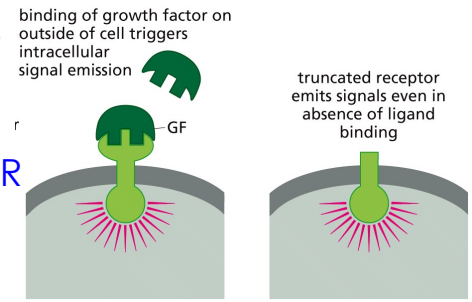


Proto-oncogene activation: Point mutations or translocations – lead to truncation or fusion proteins

Mutations (point mutation or translocation) can lead to a loss of regulation.

- "false" stop codons can be added or pauses in mRNA reading frames lead to truncated proteins
- Examples include EGFR



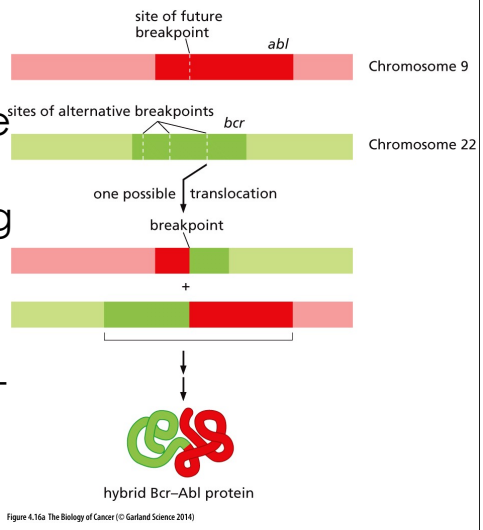


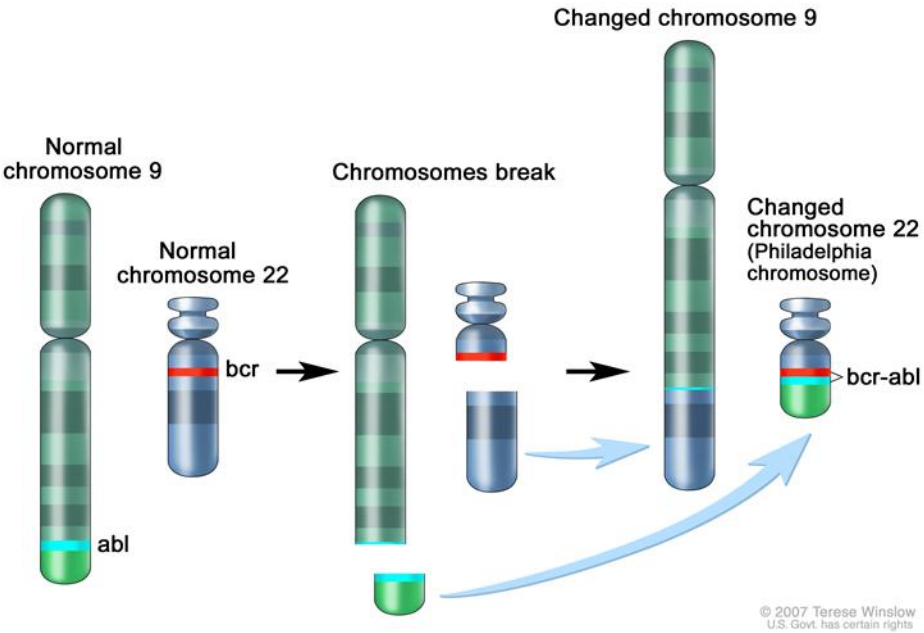
Translocaton within genes produce new oncogene

Two proto-oncogenes are "mixed and matched" to generate unregulated kinase expressed in the wrong tissue

- Abl and Bcr

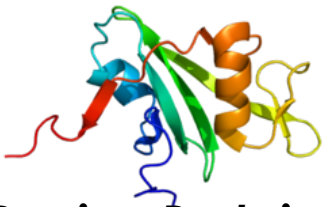
Imatinib/GLEEVEC – Bcr-Abl specific inhibitor to treat myolegenous leukemia





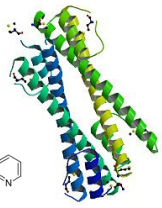
Abl – *Abelson Murine Leukemia Virus*

Tyrosine kinase (non-receptor) involved in cell division, adhesion and stress response



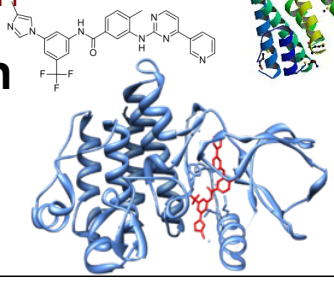
Bcr– *Break Point Cluster Region Protein*

GTPase activating protein for small G proteins with mostly unknown normal function



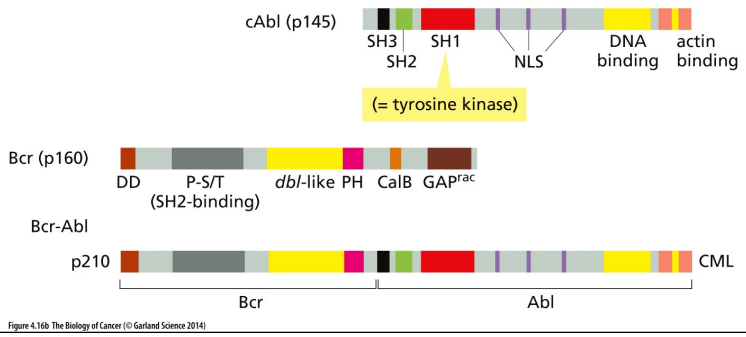
Brc-Abl Fusion Protein

Unregulated PTK activating white blood cells to grow without cytokine control



Chronic myelogenous leukemia (CML)

White blood (leukemia) cancer due to myeloid cells with extensive proliferation in bone marrow. CML leads to ~15-20% of all leukemias



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Summary

- Virus – first discovered but not primary cause of cancer
- DNA and Retroviral infection can lead to some cancer types
- Over expression of protein by amplification of gene or over expression of gene can lead to transformation
- Several mechanisms by which proto-oncogenes are activated.