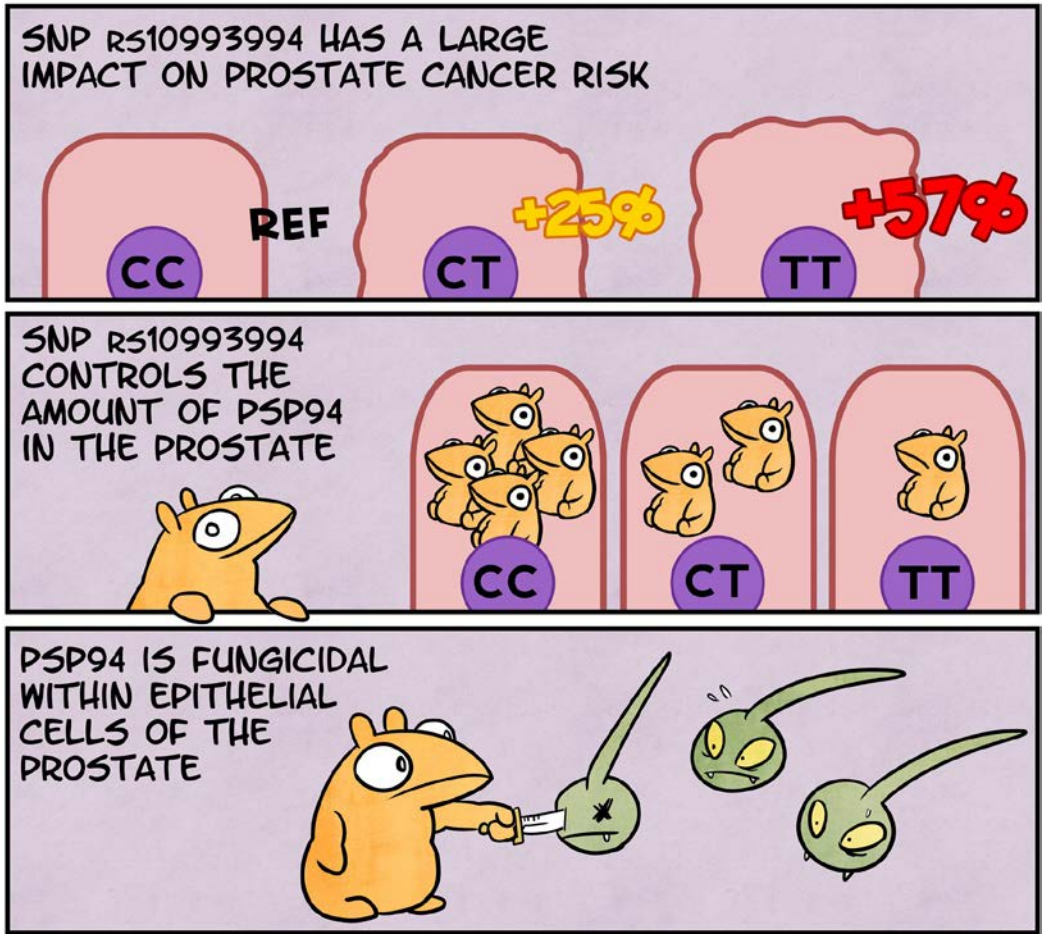
Is prostatic inflammation caused by a **chronic infection**?

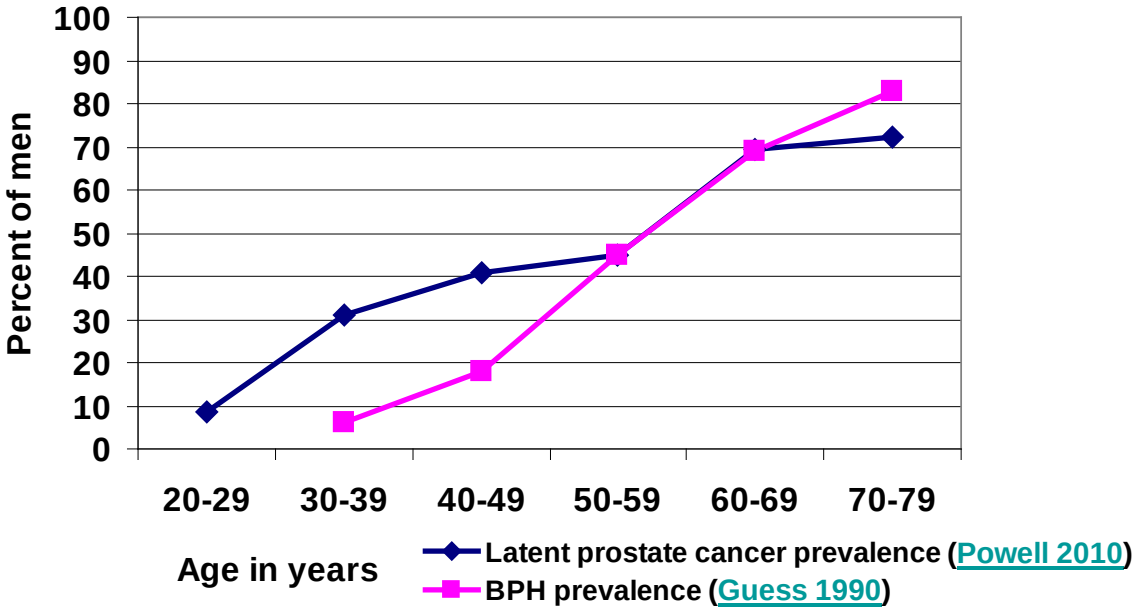


Martin Laurence, Shipshaw Labs, May 2014

This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives License (v4.0 International). You are free to copy and redistribute this material, as long as it is not modified in any way or used for commercial purposes. Permissions beyond the scope of this license may be requested at: www.psp94.com/contact

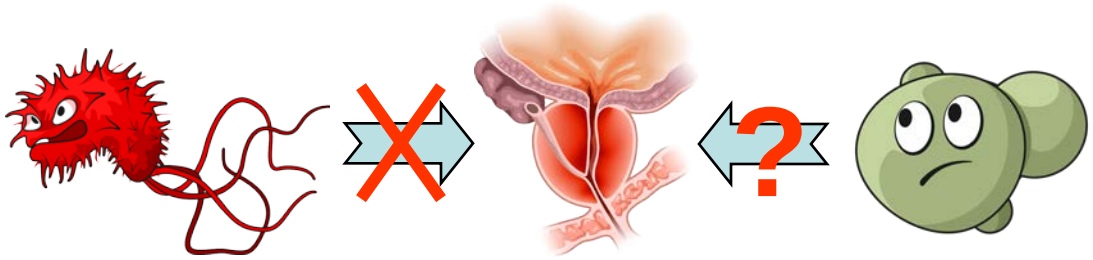
Lifetime risk of prostate disease (at age 75)

- ~10% for clinically detected prostate cancer
  - Yearly burden: **20B$** direct medical cost, **250K deaths**
- **~70% for latent prostate cancer**
  - Detected at autopsy after accidental death
- **~80% for BPH** (benign prostatic hyperplasia)
  - Detected at autopsy



Prostatic inflammation is **ubiquitous**

- Prostatic inflammation can be detected in:
  - **~20%** of 20 year old men's semen
  - **~60%** of 40 year old men's prostatic fluid
  - **~80%** of 60 year old men's prostate biopsy tissue
- Prostatic inflammation is suspected of **causing prostate cancer** and BPH (De Marzo 2007)
- Cause of prostatic inflammation itself is not known
  - Researchers have been looking for **causative bacteria/viruses** for decades, but **have not found a link** with the main diseases of the prostate
- **No systematic search of infectious agents has ever been published**
  - Many unknown fastidious fungal species have recently been shown to colonize humans (Paulino 2008, Ghannoum 2010), yet no studies have searched for fastidious fungi in the prostate



Detection method (Hrbacek 2012)	# of studies
ISH/IHC/IF/Other	14
PCR (species specific)	58
PCR (consensus bacteria)	3
PCR (consensus fungi)	0
Unbiased high-throughput sequencing (truly universal)	0

Does prostate cancer have an **infectious etiology**?

Strickler 2001 – Sexual risk factors = undetected microbe

"The situation has similarities to that observed for cervical cancer before the association with human papillomavirus was understood: a moderate association was found with sexual behavior and occasional weak associations were found with herpes simplex virus or other sexually transmitted infections, each acting as surrogates for human papillomavirus. By analogy, the findings reviewed above could reflect a **yet unrecognized sexually transmitted infection etiologically related to a subset of prostate cancer**."

De Marzo 2007 – Inflammation = undetected microbe

"Because many additional bacterial sequences, and now a new viral sequence, can be found in prostate tissue in the absence of an ability to culture any of these organisms using traditional means, it is still possible that in analogy to *H. pylori* gastritis, **researchers have missed a previously unidentified pathogen associated with most inflammatory lesions in the prostate**."

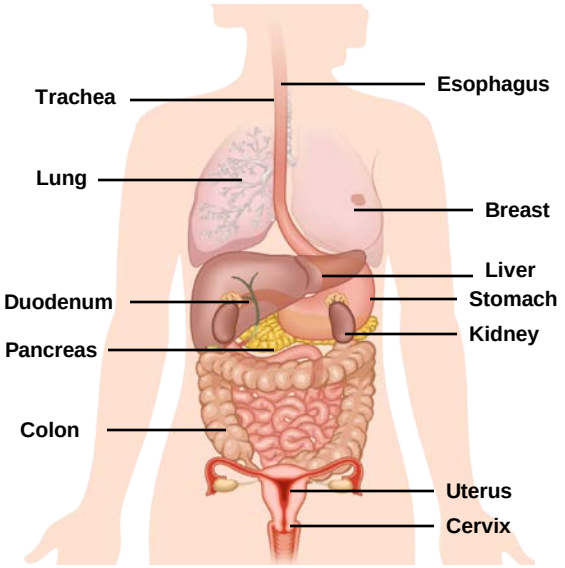
Wright 2012 – Sexual risk factors are real

"**We disagree with their suggestion that the evidence is "tenuous" between sexually transmitted infections (STIs) and the risk of PCa**. As we describe in our article, a meta-analysis of 29 studies found a summary relative risk of 1.5 (95% confidence interval [95% CI], 1.3-1.7) for PCa in men with a history of STIs, several STIs have been found in the prostate, and higher serum levels of antibodies (herpes simplex virus, Trichomonas) and prostate human papillomavirus DNA have been associated with PCa."

- Based on **sexual risk factors**, epidemiologists have predicted that cervical cancer and prostate cancer are caused by **yet-to-be-identified sexually transmitted infections (STIs)**
  - They were right about cervical cancer (HPV causes >90% of cases)
  - Are they right about prostate cancer?

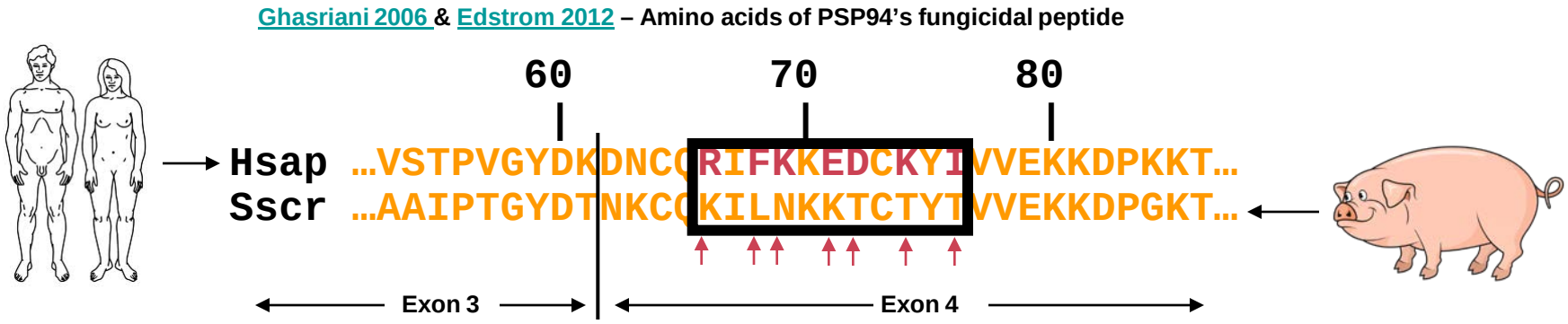
Prostate secretory protein 94 (PSP94)

- PSP94 is one of the three main proteins secreted by prostatic epithelial cells
  - PAP: ~3 mg/ml (prostatic acid phosphatase)
  - PSA: ~3 mg/ml (prostate-specific antigen)
  - PSP94: ~2 mg/ml (prostate secretory protein 94)
    - 2σ range is **0.5 mg/ml to 8.0 mg/ml**
- PSP94 is **present in** epithelial cells of **many organs** in both men and women
  - Prostate, breast
  - Uterus, cervix
  - Lung, trachea, esophagus
  - Colon, stomach, duodenum
  - Liver, pancreas, kidney
- PSP94 is **evolving very rapidly**



In 2012, Anneli Edstrom & Ole Sorensen discovered that PSP94 was highly fungicidal

- PSP94 **disrupts** plasma membranes containing **ergosterol**
- Its structure is almost identical in humans and pigs
  - Fungicidal peptide is in exon 4 (black rectangle, below)
- A **fungal pathogen** is likely driving PSP94's **rapid evolution**
  - Fungicidal peptide has only **4 out of 11 amino acids** in common between pig and human versions, yet both are **highly fungicidal**
- Fungicidal activity is thus a primary function of PSP94



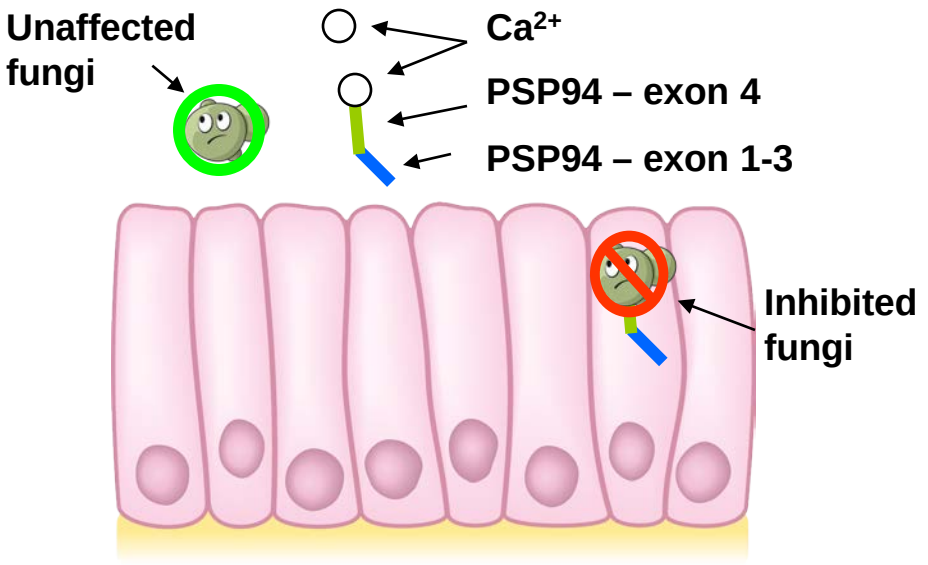
PSP94 fungicidal activity is inhibited by calcium ions

Edstrom 2012 – Calcium ions inhibit PSP94's fungicidal activity

	Ca^{2+} (uM)		PSP94 (uM)	Fungi-cidal?	pH
Prostatic secretion	18000	>	250	**No**	6.2 – 8.0
Seminal plasma	7000	>	62	**No**	7.2 – 8.0
Epithelial cells	0.2	<	Much more	**Yes**	7.4

Calcium ions in prostatic secretion and seminal plasma bind to PSP94 and inhibit fungicidal activity

Fungicidal activity is only possible **within epithelial cells**, thus PSP94 targets an **intracellular fungal pathogen**



Low PSP94 concentration substantially increases prostate cancer risk

Eeles 2008 – SNP rs10993994 strongly affects prostate cancer risk

"The most strongly associated SNP, rs10993994, is 2 bp upstream of the transcription start site of *MSMB*. Its location and the strength of the association raises the possibility that this SNP may be **causally related to [prostate cancer] risk**, but resequencing and further analyses will be needed clarify the functional basis of this association."

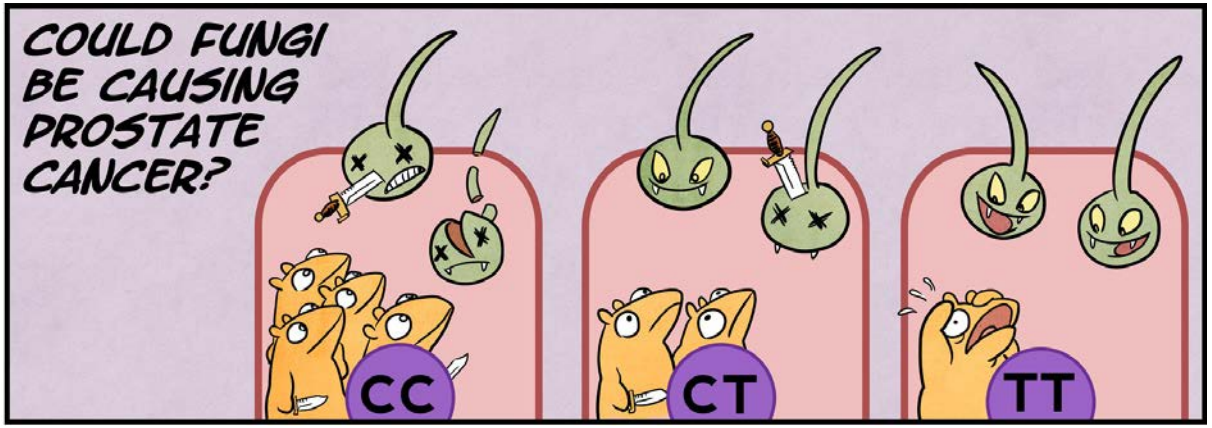
PSP94 concentration controlled by SNP rs10993994 (C=high; T=low)

The *MSMB* gene encodes PSP94

Low PSP94 concentration makes prostate epithelial cells more suitable for fungal growth, increasing inflammation

Low PSP94 concentration is one of the most important **genetic risk factors** of prostate cancer

SNP rs10993994	PSP94 concentration ratio (Xu 2010)	Prostate cancer odds ratio (Thomas 2008)
CC	1.00	1.00x
CT	0.63	1.24x
TT	0.31	1.66x



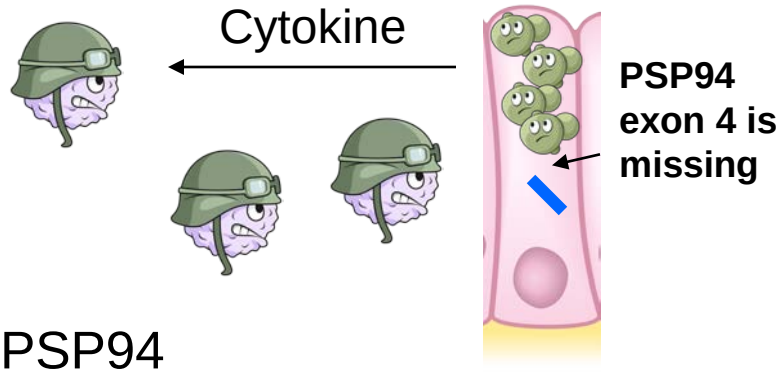
Truncated PSP94 is a biomarker of BPH

Kramer 2006 – How BPH develops: T cells kill epithelial cells

> **"When local accumulation of T cells reaches a certain threshold, surrounding [epithelial] cells become targets and are killed either specifically or in bystander reactions, leaving behind vacant spaces that are replaced by fibromuscular nodules with a specific pattern of a Th0/Th3 type of immune response**. Currently, we do not have a concept as to why the leukocyte population increases in BPH, whether it is an abnormal response to a physiological stimulus or induced by a chronic pathological stimulus."

Xu 2003 – PSP94 w/o fungicidal region is a biomarker of BPH

> "Most importantly, **we identified a modified isoform of the human prostate secretory protein [PSP94], i.e., PSP61, which was uniquely expressed in EPS from BPH patients but undetectable in the normal control**. Our results indicate that PSP61 may have a great potential as a specific marker for detecting BPH."

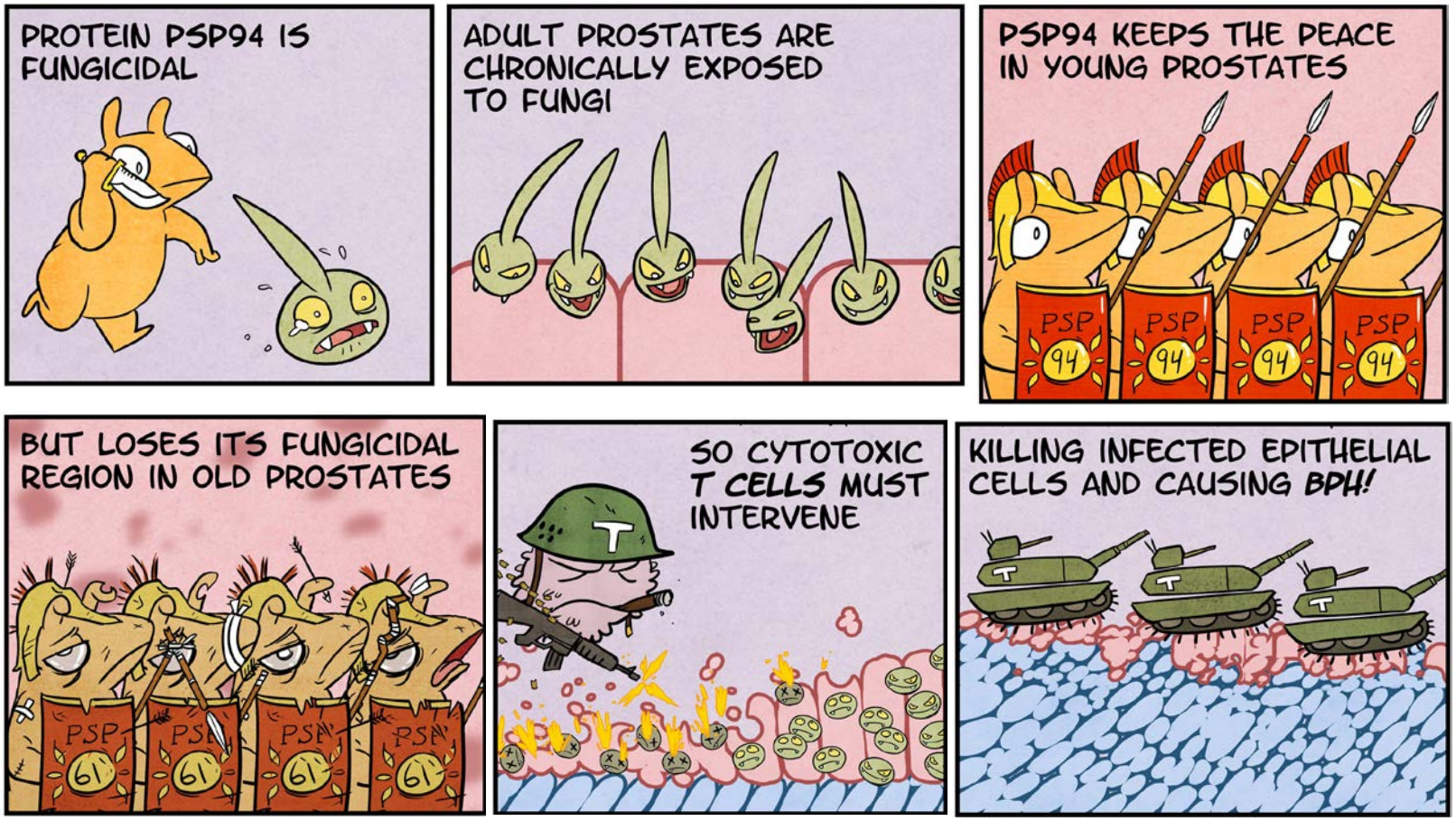


Men who secrete a truncated version of PSP94 **which lacks fungicidal region have BPH**

TCR γ/δ **T cells** known to respond to intracellular microorganisms **increase markedly in BPH**

This strongly suggests that **BPH is caused by a highly prevalent intracellular fungal pathogen**

Depiction of events leading to BPH



Are diseases of the prostate caused by a **chronic infection**?

- **Yes**, this is quite likely
  - BPH and prostate cancer are probably caused by an **intracellular microorganism with ergosterol** in its plasma membrane
- What can we do about it?
  - Publish a review article about PSP94 and prostate cancer
    - Status: **done** (by our group, Sutcliffe 2014)
  - Publish a review article about PSP94 and BPH
    - Status: **work in progress**
  - Test hypothesis using epidemiological data
    - Status: **done** (by an independent group, Stott-Miller 2013)
  - **Identify the causative microorganism**
    - Status: **pending** (methods and preliminary results, Laurence 2014)
  - **Kill the causative microorganism** with ergosterol targeting drugs (such as terbinafine and itraconazole)
    - Status: work dependent on microorganism identification