The urothelium is a critical barrier between the urine and blood.
The urothelium is a critical barrier between the blood and urine

The urothelium is lined with superficial cells that are specialized for synthesis and transport of uroplakins, a family of proteins that assemble into a crystalline apical barrier.

The urothelium is one of the most quiescent epithelia in the body.

The urothelium contains progenitors that can regenerate superficial cells in adults after injury from toxins or urinary tract infections.
## Features of Adult Urothelial Cells

<table>
<thead>
<tr>
<th>Superficial:</th>
<th>Krt20+ Upk+ P63- Krt5- Krt14-</th>
<th>Binucleated 4n+4n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermediate:</td>
<td>Krt20- Upk+ P63+ Krt5- Krt14-</td>
<td>Binucleated 2n+2n</td>
</tr>
<tr>
<td>Intermediate:</td>
<td>Krt20- Upk+ P63+ Krt5- Krt14-</td>
<td>Mononucleated 2n</td>
</tr>
<tr>
<td>K5-Basal:</td>
<td>Krt20- UpkLow P63+ Krt5+ Krt14-</td>
<td></td>
</tr>
<tr>
<td>K14-Basal:</td>
<td>Krt20- Upk- P63+ Krt5+ Krt14+</td>
<td>2 Basal Cell Populations</td>
</tr>
</tbody>
</table>
What cell types are urothelial progenitors?
UROTHELIAL REGENERATION INDUCED BY INFECTION WITH E.COLI STRAIN UTI 89

UROTHELIAL INNATE IMMUNE RESPONSE

IBCS AND EXFOLIATION

PROLIFERATION/REGENERATION
INTERMEDIATE CELLS ARE SUPERFICIAL CELL PROGENITORS

Krt5CreERT2; mTmG

UPK3AGCE; MCHERRY

KRT20 RFP SURVIVIN CEN

INTERMEDIATE AND SUPERFICIAL CELL DAUGHTER IN TELOPHASE

TAMOX ECOLI EDU HARVEST
- Basal cells self-renew but produce few superficial cell daughters after 1 round of CPP or UTI induced regeneration.

- They undergo dominant symmetrical divisions.
How are binucleate intermediate cells produced?
And how are superficial cells produced?

- Failed cytokinesis
- Endoreplication
- Fusion
TIME LAPSE SHOWING FAILED CYTOKINESIS

UPK2CREERT2;MTMG X UP2CREERT2;NTNG

** Failed Cytokinesis

2n+2n → Endoreplication → 4n+4n

Fusion
No evidence → 2n+2n

Diagram showing the stages of failed cytokinesis and the processes of endoreplication and fusion.
Intermediate cells undergo failed cytokinesis to produce binucleate superficial cells.
BINUCLEATE 2N+2N INTERMEDIATE ENTER S-PHASE BUT ARE RARELY IF EVER FOUND IN METAPHASE, ANAPHASE OR TELEPHASE

4N+4N SUPERFICIAL CELLS FORM VIA ENDOREPLICATION
“NORMAL” PATHWAY OF UROTHELIAL REGENERATION

Intermediate cells undergo symmetric divisions and also undergo failed cytokinesis to generate binucleate superficial cells.

Binucleate intermediate cells give rise to superficial cells via endoreplication.

connection to BM is lost

Failed cytokinesis

progenitor

endoreplication

4n x 2

2n x 2

Superficial cell

failed cytokinesis

endoreplication

2n x 2
KRT14 marks a subpopulation of bladder basal cells with pivotal role in regeneration and tumorigenesis. Papafotiou, et al 2016

**UTI-BASAL CELLS PRODUCE BASAL CELLS**

![Image of cellular staining with labels](image)

1X or 5X cyclophosphamide

**WHEN DO K14 BASAL CELLS PRODUCE SUPERFICIAL CELLS?**
BASAL CELLS CAN PRODUCE INTERMEDIATE CELLS AND SUPERFICIAL CELLS AFTER CHRONIC DAMAGE

Intermediate cell

Basal cell

Superficial cell

Cyclophosphamide is metabolized to acrolein, a carcinogen found in tobacco – induces DNA damage

Repeated or high doses of cyclophosphamide induce edema and inflammation

Model of interstitial cystitis

Vera et al., 2009
Urothelial cells have features that may be relevant to tumorigenesis.

**UROTHELIUM AT HOMEOSTASIS**
DNA Damage-Polyplody related?

**UROTHELIUM AFTER UTI-NFKB/TLR SIGNALING**
(Tumor cells may have innate/adaptive immune functions)

**NO BBN**

**BBN 1 month**

Granulation tissue and edema

AFTER CPP, BBN
POTENTIAL OF UROTHELIAL CELL TYPES

K5/K14 Basal cells

Intermediate cells
Superficial cells

ACUTE INJURY (UTI)

CHRONIC INJURY

MIBC
Papillary Lesions
Squamous Lesions
CIS

MIBC
Papillary Lesions
Squamous Lesions
CIS